



PCT/NZ03/00105

CERTIFICATE

I hereby certify that annexed is a true copy of the PCT International Application
PCT/NZ03/00105 filed on 27 May 2003 in the name of Genesis Research and Development
Corporation Limited.

Dated 31 August 2005.

Neville Harris
Commissioner of Patents, Trade Marks and Designs



PCT

REQUEST

The undersigned requests that the present international applications be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

PCT/NZ03 0 0 1 0 5

International Filing Date

27 MAY 2003 (27/05/03)

NEW ZEALAND PATENT OFFICE

Name of receiving Office and the International Application

Applicant's or agent's file reference
(if desired) (12 characters maximum)
JC219117/142

Box No. I TITLE OF INVENTION

FIBROBLAST GROWTH FACTOR RECEPTORS AND METHODS FOR THEIR USE

Box No. II APPLICANT

☐

This person is also inventor

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

GENESIS RESEARCH AND DEVELOPMENT CORPORATION LIMITED

1 Fox Street

Parnell

Auckland

New Zealand

Telephone No. 0064 4 373 5600

Facsimile No. 0064 4 373 5601

Teleprinter No.

Applicant's registration No. with the Office

State (that is, country) of nationality: New Zealand

State (that is, country) of residence: New Zealand

This person is applicant
for the purposes of:

☐

all designated
States

☒

all designated States
except the United States
of America

☐

the United States
of America only

☐

the States indicated in
the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

MURISON, James G

24 Calgary Street

Sandringham

Auckland

New Zealand

This person is

☐

applicant only

☒

applicant and inventor

☐

inventor only (If this check-box is
marked, do not fill in below)

Applicant's registration No. with the Office

State (that is, country) of nationality: New Zealand

State (that is, country) of residence: New Zealand

This person is applicant
for the purposes of:

☐

all designated
States

☐

all designated States
except the United States
of America

☒

the United States
of America only

☐

the States indicated in
the Supplemental Box

☒

Further applicant and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:

☒

agent

☐

common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country)

BALDWIN SHELSTON WATERS

P O Box 852, Wellington

NEW ZEALAND

Telephone (04) 472 1094

Facsimile No. (04) 473 6712

Teleprinter No.

Agent's registration No. with the Office

☐

Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

SLEEMAN, Matthew
Church Barn
Weston Colville
Cambridgeshire CB1 5PH
United Kingdom

This person is

- ☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below)

Applicant's registration No. with the Office

State (that is, country) of nationality: United Kingdom

State (that is, country) of residence: United Kingdom

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

This person is

- ☐ applicant only
☐ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below)

Applicant's registration No. with the Office

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

This person is

- ☐ applicant only
☐ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below)

Applicant's registration No. with the Office

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

This person is

- ☐ applicant only
☐ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below)

Applicant's registration No. with the Office

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box



Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No.V DESIGNATION OF STATES

Mark the applicable check-boxes below; at least one must be marked.

The following designations are hereby made under Rule 4.9(a):

Regional Patent

- ☒ **AP** **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZA Zambia, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT (if other kind of protection or treatment desired, specify on dotted line):
- ☒ **EA** **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** **European Patent:** AT Austria, BE Belgium, BG Bulgaria, CH and LI Switzerland and Liechtenstein, CY Cyprus, CZ Czech Republic, DE Germany, DK Denmark, EE Estonia, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, RO Romania, SE Sweden, SI Slovenia, SK Slovakia, TR Turkey, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA** **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GQ Equatorial Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line):

National Patent (if other kind of protection or treatment desired, specify on dotted line):

- | | | |
|--|---|--|
| <input checked="" type="checkbox"/> AE United Arab Emirates | <input checked="" type="checkbox"/> GM Gambia | <input checked="" type="checkbox"/> NZ New Zealand |
| <input checked="" type="checkbox"/> AG Antigua and Barbuda | <input checked="" type="checkbox"/> HR Croatia | <input checked="" type="checkbox"/> OM Oman |
| <input checked="" type="checkbox"/> AL Albania | <input checked="" type="checkbox"/> HU Hungary | <input checked="" type="checkbox"/> PH Philippines |
| <input checked="" type="checkbox"/> AM Armenia | <input checked="" type="checkbox"/> ID Indonesia | <input checked="" type="checkbox"/> PL Poland |
| <input checked="" type="checkbox"/> AT Austria (and Utility Model) | <input checked="" type="checkbox"/> IL Israel | <input checked="" type="checkbox"/> PT Portugal |
| <input checked="" type="checkbox"/> AU Australia | <input checked="" type="checkbox"/> IN India | <input checked="" type="checkbox"/> RO Romania |
| <input checked="" type="checkbox"/> AZ Azerbaijan | <input checked="" type="checkbox"/> IS Iceland | <input checked="" type="checkbox"/> RU Russian Federation |
| <input checked="" type="checkbox"/> BA Bosnia and Herzegovina | <input checked="" type="checkbox"/> JP Japan | <input checked="" type="checkbox"/> SC Seychelles |
| <input checked="" type="checkbox"/> BB Barbados | <input checked="" type="checkbox"/> KE Kenya | <input checked="" type="checkbox"/> SD Sudan |
| <input checked="" type="checkbox"/> BG Bulgaria | <input checked="" type="checkbox"/> KG Kyrgyzstan | <input checked="" type="checkbox"/> SE Sweden |
| <input checked="" type="checkbox"/> BR Brazil | <input checked="" type="checkbox"/> KP Democratic People's Republic Of Korea | <input checked="" type="checkbox"/> SG Singapore |
| <input checked="" type="checkbox"/> BY Belarus | <input checked="" type="checkbox"/> KR Republic of Korea | |
| <input checked="" type="checkbox"/> BZ Belize | <input checked="" type="checkbox"/> KZ Kazakhstan | <input checked="" type="checkbox"/> SK Slovakia (and Utility Model) |
| <input checked="" type="checkbox"/> CA Canada | <input checked="" type="checkbox"/> LC Saint Lucia | <input checked="" type="checkbox"/> SL Sierra Leone |
| <input checked="" type="checkbox"/> CH & LI Switzerland and Liechtenstein | <input checked="" type="checkbox"/> LK Sri Lanka | <input checked="" type="checkbox"/> TJ Tajikistan |
| <input checked="" type="checkbox"/> CN China | <input checked="" type="checkbox"/> LR Liberia | <input checked="" type="checkbox"/> TM Turkmenistan |
| <input checked="" type="checkbox"/> CO Colombia | <input checked="" type="checkbox"/> LS Lesotho | <input checked="" type="checkbox"/> TN Tunisia |
| <input checked="" type="checkbox"/> CR Costa Rica | <input checked="" type="checkbox"/> LT Lithuania | <input checked="" type="checkbox"/> TR Turkey |
| <input checked="" type="checkbox"/> CU Cuba | <input checked="" type="checkbox"/> LU Luxembourg | <input checked="" type="checkbox"/> TT Trinidad and Tobago |
| <input checked="" type="checkbox"/> CZ Czech Republic (and Utility Model) | <input checked="" type="checkbox"/> LV Latvia | <input checked="" type="checkbox"/> TZ United Republic of Tanzania |
| <input checked="" type="checkbox"/> DE Germany (and Utility Model)..... | <input checked="" type="checkbox"/> MA Morocco | <input checked="" type="checkbox"/> UA Ukraine |
| <input checked="" type="checkbox"/> DK Denmark (and Utility Model)..... | <input checked="" type="checkbox"/> MD Republic of Moldova | <input checked="" type="checkbox"/> UG Uganda |
| <input checked="" type="checkbox"/> DM Dominica | <input checked="" type="checkbox"/> MG Madagascar | <input checked="" type="checkbox"/> US United States of America..... |
| <input checked="" type="checkbox"/> DZ Algeria | <input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia | <input checked="" type="checkbox"/> UZ Uzbekistan |
| <input checked="" type="checkbox"/> EC Ecuador | | <input checked="" type="checkbox"/> VC Saint Vincent and The Grenadines |
| <input checked="" type="checkbox"/> EE Estonia (and Utility Model) | | <input checked="" type="checkbox"/> VN Viet Nam |
| <input checked="" type="checkbox"/> ES Spain | <input checked="" type="checkbox"/> MN Mongolia | |
| <input checked="" type="checkbox"/> FI Finland (and Utility Model) | <input checked="" type="checkbox"/> MW Malawi | <input checked="" type="checkbox"/> YU Serbia and Montenegro |
| <input checked="" type="checkbox"/> GB United Kingdom | <input checked="" type="checkbox"/> MX Mexico | <input checked="" type="checkbox"/> ZA South Africa |
| <input checked="" type="checkbox"/> GD Grenada | <input checked="" type="checkbox"/> MZ Mozambique | <input checked="" type="checkbox"/> ZM Zambia |
| <input checked="" type="checkbox"/> GE Georgia | <input checked="" type="checkbox"/> NI Nicaragua | <input checked="" type="checkbox"/> ZW Zimbabwe |
| <input checked="" type="checkbox"/> GH Ghana | <input checked="" type="checkbox"/> NO Norway | |
| <input checked="" type="checkbox"/> FI Finland | | |

Check-boxes below reserved for designating States which have become party to the PCT after issuance of this sheet:

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation (including fees) must reach the receiving Office within the 15-month limit).

Box No. VI PRIORITY CLAIM

priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application (day/month/year)	Number of earlier application	National application: Country or Member of WTO	Where earlier application is:	
			regional application:* regional Office	international application: receiving Office
item (1) 28 May 2002 (28/05/02)	US 10/157,444	United States of America		
Item (2)				
item (3)				
item (4)				
item (5)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (*only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office*) identified above as:

☐ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ item (4) ☐ item (5) ☐ other, see
Supplemental Box

*Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)).

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):

ISA /AU

Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):

Date (day/month/year) Number Country (or regional Office)

Box No. VIII DECLARATIONS

The following declarations are contained in Boxes Nos. VIII (i) to (v) (mark the applicable Check-boxes below and indicate in the right column the number of each type of declaration):

Number of
Declarations

- | | | | |
|--------------------------|--------------------|--|---|
| <input type="checkbox"/> | Box No. VIII (i) | Declaration as to the identity of the inventor | : |
| <input type="checkbox"/> | Box No. VIII (ii) | Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent | : |
| <input type="checkbox"/> | Box No. VIII (iii) | Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application | : |
| <input type="checkbox"/> | Box No. VIII (iv) | Declaration of inventorship (only for the purposes of the designation of the United States of America) | : |
| <input type="checkbox"/> | Box No. VIII (v) | Declaration as to non-prejudicial disclosures or exceptions to lack of novelty | : |

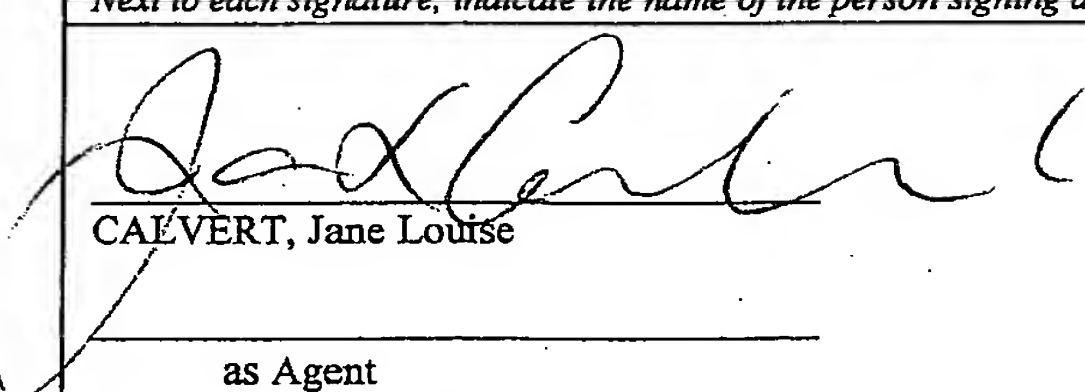
Box No. IX CHECK LIST; LANGUAGE OF FILING

This international application contains: the following number of sheets in the paper form:		This international application is accompanied by the following item(s) (mark the applicable check-boxes below and indicate in right column the number of each item):		Number of items
request (including declaration sheets)	5	1. <input checked="" type="checkbox"/> fee calculation sheet	:	
Description (excluding sequence listing part)	61	2. <input type="checkbox"/> original separate power of attorney	:	
Claims	9	3. <input type="checkbox"/> original general power of attorney:	:	
Abstract	1	4. <input type="checkbox"/> copy general power of attorney; reference number; if any:	:	
Drawings	11	5. <input type="checkbox"/> statement explaining lack of signature	:	
Sub-total number of sheets	87	6. <input type="checkbox"/> priority document(s) identified in Box No. VI as item(s);	:	
sequence listings	:	7. <input type="checkbox"/> translation of international application into (language);.....	:	
tables related thereto	:	8. <input type="checkbox"/> separate indications concerning deposited microorganism or other biological material	:	
(for both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (c) below)	9	9. sequence listing in computer readable form (indicate also type and number of carriers) (diskette, CD-ROM, CD-R or other):	:	1
Total number of sheets	96	(i) <input checked="" type="checkbox"/> copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application)	:	
(b) <input type="checkbox"/> only in computer readable form (under Section 801(a)(i))		(ii) <input type="checkbox"/> (only where check-box (b)(i) or (b)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter	:	
(i) <input type="checkbox"/> sequence listings		(iii) <input checked="" type="checkbox"/> together with relevant statement as to the identity of the copy or copies with the sequence listing mentioned in left column	:	1
(ii) <input type="checkbox"/> tables related thereto		10. <input type="checkbox"/> tables in computer readable form related to sequence listings (indicate type and number of carriers)	:	
(c) <input type="checkbox"/> also in computer readable form (under Section 801(a)(ii))		(i) <input type="checkbox"/> copy submitted for the purposes of international search under Section 802(b-quater) only (and not as part of the international application)	:	
(i) <input type="checkbox"/> sequence listings		(ii) <input type="checkbox"/> (only where check-box (b)(ii) or (c)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Section Section 802(b-quater)	:	
(ii) <input type="checkbox"/> tables related thereto		(iii) <input type="checkbox"/> together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column	:	
Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the		11. <input type="checkbox"/> other (specify):.....	:	
<input type="checkbox"/> sequence listings				
<input type="checkbox"/> tables related thereto.....				
(additional copies to be indicated under items 9 (ii), and/or 10(ii))				

Figure of the drawings which
should accompany the abstract:Language of filing of the
international application: English

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).


 CALVERT, Jane Louise

as Agent

For receiving Office use only		2. Drawings:	
1. Date of actual receipt of the purported international application:	27 MAY 2003 (27/05/03)	<input type="checkbox"/> received:	
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:		<input type="checkbox"/> not received:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):			
5. International Searching Authority (if two or more are competent):	ISA/AU	6. <input type="checkbox"/> Transmittal of search copy delayed until search fee if paid	

Date of receipt of the record copy
by International Bureau:

For International Bureau use only

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET
Annex to the Request

For receiving Office use only

International Application No **PCT/NZ030 0 1 0 5**

Date stamp of the receiving Office **27 MAY 2003 (27/05/03)**

Applicant's or Agent's
File reference:

JC219117/142

Applicant **GENESIS RESEARCH AND DEVELOPMENT CORPORATION LIMITED**

CALCULATION OR PRESCRIBED FEES

1. TRANSMITTAL FEE

\$180.00

T

2. SEARCH FEE

\$1,073.00

S

International search to be carried out by AU
(if two or more International Searching Authorities are competent to carry out the international search, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE
Basic Fee

Where item (b) of Box No. IX applies, enter Sub-total number of sheets
Where item (b) of Box No. IX does not apply, enter Total number of sheets 96

b1 first 30 sheets

\$890.00

b1

b2

66
number of sheets
in excess of 30

x

\$20.00
fee per sheet

= 1,320.00

b2

b3

additional component (only if sequence listings and or tables related thereto are filed in computer readable form under Section 801 (a)(i), or both in that form and on paper, under Section 801(a)(ii):

400 x \$20.00 = 0.00
fee per sheet

b3

Add amounts entered at b1, b2 and b3 and enter total at B

\$2,210.00

B

Designation Fees

The international application contains 118 designations.

maximum x \$192.00
Number of designation fees payable (maximum 5) amount of designation fee

\$960.00

D

Add amounts entered at B and D and enter total at I
(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

\$3,170.00

I

4. FEE FOR PRIORITY DOCUMENT (if applicable)

\$0.00

P

5. TOTAL FEES PAYABLE

\$4,423.00

Add amounts entered at T, S, I and P, and enter total in TOTAL box

TOTAL

☐ The designation fees are not paid at this time.

MODE OF PAYMENT

☐ authorization to charge
..... deposit account (see below)

☐ postal money order

☐ cash

☐ coupons

☒ cheque

☐ bank draft

☐ revenue stamps

☐ other (specify):

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT
(this mode of payment may not be available at all receiving Offices)

Receiving Office: RO/

☐ Authorization to charge the total fees indicated above.

Deposit Account No. _____

☐ (This check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit) Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.

Date: _____

☐ Authorization to charge the fee for priority document.

Name: _____

Signature: _____

COPY

PCT/NZ03/00105

PCT

GENERAL POWER OF ATTORNEY

(for several international applications filed under the Patent Cooperation Treaty)

(PCT Rule 90.5)

The undersigned person(s) :

(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

GENESIS RESEARCH & DEVELOPMENT CORPORATION

1 Fox Street

Parnell

Auckland

New Zealand

hereby appoint(s) the following person as:



agent



common representative

Name and address

(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

HAWKINS, MICHAEL HOWARD

TERRY, JOHN KINNEAR

THOREAU, PHILIP AUBYN BERTRAM

PAIRMAN, JANE ELIZABETH

MONK, JONATHAN PAUL

JACKSON, TIMOTHY GRAHAM

LYTH, RICHARD JOHN

BALLANCE, JULIE ANNE

LYNCH, GREGORY PAUL

CALVERT, JANE LOUISE

SUTTON, ANGELA AITCHISON

BALDWIN SHELSTON WATERS

NCR Building

342 Lambton Quay

Wellington 6001

New Zealand

to represent the undersigned before



all the competent International Authorities



the International Searching Authority only



the International Preliminary Examining Authority only

in connection with any and all international applications filed by the undersigned with the following Office

New Zealand PCT Receiving Office

as receiving Office

and to make or receive payments on behalf of the undersigned.

Signature(s) (where there are several persons, each of them must sign; next to each signature, indicate the name of the person signing and the capacity in which the person signs, if such capacity is not obvious from reading this power):



J. D. Watson, CEO

~~Elizabeth Visser, Patent Manager~~

Date:

4 December 2000

FIBROBLAST GROWTH FACTOR RECEPTORS AND METHODS FOR THEIR USE

Cross-Reference to Related Applications

5 This application claims priority to U.S. Patent Application No. 10/157,444, filed May 28, 2002, which is a continuation-in-part of U.S. Patent Application No. 09/823,038, filed March 28, 2001, which claims priority to International Patent Application No. PCT/NZ00/00015, filed February 18, 2000; and to U.S. Provisional Patent Application No. 60/221,216, filed July 25, 2000, and is a continuation-in-part of U.S. Patent
10 Application No. 09/383,586, filed August 26, 1999, now U.S. Patent 6,424,419; which is a continuation-in-part of U.S. Patent Application No. 09/276,268, filed March 25, 1999, now abandoned.

Technical Field of the Invention

15 This invention relates to polynucleotides and polypeptides derived from lymph node stromal cells from flaky skin (*fsn* ^{-/-}) mice, human homologues of such polynucleotides, polypeptides, and antibodies and other molecules that specifically bind to the inventive polypeptides, and the use of such polynucleotides, polypeptides, and binding molecules in therapeutic and diagnostic methods. Specific binding molecules
20 include antibodies, functional fragments thereof, as well as scFv and *Camelidae* heavy chain IgG that specifically bind to FGFR5 thereby modulating the activity of FGFR5. Thus, specific binding molecules encompass agonists and/or antagonists of FGFR5 activity that are effective agents suitable for the treatment of diseases such as osteopontin-mediated autoimmune disease, such as systemic lupus erythematosus, bone
25 disorders including osteoporosis and osteopetrosis, and cancers, including cellular carcinomas such as hepatocellular carcinomas.

Background of the Invention

30 Lymph vessels and nodes are important components of the body's immune system. Lymph nodes are small lymphatic organs that are located in the path of lymph vessels. Large molecules and cells, including foreign substances, enter into the lymphatic

vessels and, in circulating through these vessels, pass through the lymph nodes. Here, any foreign substances are concentrated and exposed to lymphocytes. This triggers a cascade of events that constitute an immune response, protecting the body from infection and from cancer.

5 Lymph nodes are surrounded by a dense connective tissue network that forms a supporting capsule. This network extends into the body of the lymph node, forming an additional framework of support. Throughout the remainder of the organ, a fine meshwork can be identified that comprises reticular fibres and the reticular cells that produce and surround the fibres. These features provide a support for the main functional
10 cells of the lymphatic system, which are T- and B-lymphocytes. Additional cell types found in lymph nodes include macrophages, follicular dendritic cells, and endothelial cells that line the blood vessels servicing the node.

 The cells within lymph nodes communicate with each other in order to defend the body against foreign substances. When a foreign substance, or antigen, is present, it is
15 detected by macrophages and follicular dendritic cells that take up and process the antigen, and display parts of it on their cell surface. These cell surface antigens are then presented to T- and B-lymphocytes, causing them to proliferate and differentiate into activated T-lymphocytes and plasma cells, respectively. These cells are released into the circulation in order to seek out and destroy antigen. Some T- and B-lymphocytes will
20 also differentiate into memory cells. Should these cells come across the same antigen at a later date, the immune response will be more rapid.

 Once activated T- and B-lymphocytes are released into the circulation, they can perform a variety of functions that lead to the eventual destruction of antigen. Activated T-lymphocytes can differentiate into cytotoxic lymphocytes (also known as killer T-cells)
25 which recognise other cells that have foreign antigens on their surface and kill the cell by causing them to lyse. Activated T-lymphocytes can also differentiate into helper T-cells which will then secrete proteins in order to stimulate B-lymphocytes, and other T-lymphocytes, to respond to antigens. In addition, activated T-lymphocytes can differentiate into suppressor T-cells which secrete factors that suppress the activity of B-
30 lymphocytes. Activated B-lymphocytes differentiate into plasma cells, which synthesise and secrete antibodies that bind to foreign antigens. The antibody-antigen complex is

then detected and destroyed by macrophages, or by a group of blood constituents known as complement.

Lymph nodes can be dissociated and the resulting cells grown in culture. Cells that adhere to the tissue culture dishes can be maintained for some length of time and are known as stromal cells. The cultured cells are a heterogeneous population and can be made up of most cells residing within lymph nodes, such as reticular cells, follicular dendritic cells, macrophages and endothelial cells. It is well known that bone marrow stromal cells play a critical role in homing, growth and differentiation of hematopoietic progenitor cells. Proteins produced by stromal cells are necessary for the maintenance of plasma cells *in vitro*. Furthermore, stromal cells are known to secrete factors and present membrane-bound receptors that are necessary for the survival of lymphoma cells.

An autosomal recessive mutation, designated flaky skin (*fsn* ^{-/-}), has been described in the inbred A/J mouse strain (The Jackson Laboratory, Bar Harbour, ME). The mice have a skin disorder similar to psoriasis in humans. Psoriasis is a common disease affecting 2% of the population, which is characterised by a chronic inflammation associated with thickening and scaling of the skin. Histology of skin lesions shows increased proliferation of the cells in the epidermis, the uppermost layer of skin, together with the abnormal presence of inflammatory cells, including lymphocytes, in the dermis, the layer of skin below the epidermis. While the cause of the disease is unclear, psoriasis is associated with a disturbance of the immune system involving T lymphocytes. The disease occurs more frequently in family members, indicating the involvement of a genetic factor as well. Mice with the *fsn* gene mutation have not only a psoriatic-like skin disease but also other abnormalities involving cells of the immune and hematopoietic system. These mice have markedly increased numbers of lymphocytes associated with enlarged lymphoid organs, including the spleen and lymph nodes. In addition, their livers are enlarged, and the mice are anaemic. Genes and proteins expressed in abnormal lymph nodes of *fsn* ^{-/-} mice may thus influence the development or function of cells of the immune and hematopoietic system, the response of these cells in inflammatory disorders, and the responses of skin and other connective tissue cells to inflammatory signals.

There is a need in the art to identify genes encoding proteins that function to modulate all cells of the immune system. These proteins from normal or abnormal lymph

nodes may be useful in modifying the immune responses to tumour cells or infectious agents such as bacteria, viruses, protozoa and worms. Such proteins may also be useful in the treatment of disorders where the immune system initiates unfavourable reactions to the body, including Type I hypersensitivity reactions (such as hay fever, eczema, allergic rhinitis and asthma), and Type II hypersensitivity reactions (such as transfusion reactions and haemolytic disease of newborns). Other unfavourable reactions are initiated during Type III reactions, which are due to immune complexes forming in infected organs during persistent infection or in the lungs following repeated inhalation of materials from moulds, plants or animals, and in Type IV reactions in diseases such as leprosy, schistosomiasis and dermatitis.

Novel proteins of the immune system may also be useful in treating autoimmune diseases where the body recognises itself as foreign. Examples of such diseases include rheumatoid arthritis, Addison's disease, ulcerative colitis, dermatomyositis and lupus. Such proteins may also be useful during tissue transplantation, where the body will often recognise the transplanted tissue as foreign and attempt to kill it, and also in bone marrow transplantation when there is a high risk of graft-versus-host disease where the transplanted cells attack their host cells, often causing death.

There thus remains a need in the art for the identification and isolation of genes encoding proteins expressed in cells of the immune system for use in the development of therapeutic agents for the treatment of disorders including those associated with the immune system.

Summary of the Invention

The present invention is based upon the identification and isolation of FGFR5 polypeptides and functional portions of polypeptides expressed in lymph node stromal cells of *fsn* ^{-/-} mice and human homologues of such polypeptides, together with polynucleotides encoding such polypeptides

Thus, the present invention provides compositions comprising modulators of FGFR5 gene expression. Such modulators include, but are not limited to (a) small molecule inhibitors of gene expression, (b) anti-sense oligonucleotides, and (c) small interfering RNA molecules (siRNA or RNAi). Anti-sense oligonucleotides include (a)

anti-sense expression vectors; (b) anti-sense oligodeoxyribonucleotides, (c) anti-sense phosphorothioate oligodeoxyribonucleotides, (d) anti-sense oligoribonucleotides, and (e) anti-sense phosphorothioate oligoribonucleotides.

Within certain embodiments, modulators of FGFR5 gene expression specifically
5 bind to polynucleotides including: (a) polynucleotides comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9; (b) complements of a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9; (c) reverse sequences of a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9; (d) polynucleotides that encode a
10 polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15; (e) complements of polynucleotides that encode a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15; and (f) reverse sequences of polynucleotides that encodes a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15.

15 Within certain aspects of the present invention, the modulators of FGFR5 gene expression are effective in decreasing FGFR5 gene expression when contacted with a population of cells expressing FGFR5. Within other aspects, the modulators of FGFR5 gene expression are effective in decreasing osteopontin gene expression when contacted with a population of cells expressing FGFR5.

20 Other embodiments of the present invention provide compositions comprising binding agents wherein the binding agents are modulators of FGFR5 polypeptide function and wherein the binding agents include (a) small molecules; (b) antibodies or antigen-binding fragments thereof; (c) small chain antibody fragments (scFv); (d) camelid heavy chain antibodies (HCAb) or heavy chain variable domains thereof (V_{HH}); and (e) FGFR5
25 ligands or antigen-binding fragments thereof.

Within certain aspects of these embodiments, the binding agents specifically bind to polypeptides including (a) polypeptides encoded by a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9 or a complement thereof; and (b) polypeptides comprising a sequence selected from the group consisting
30 of: SEQ ID NO: 5-8 and 13-15.

Depending upon the precise application contemplated, binding agents may be agonists of FGFR5 polypeptide function that are, for example, effective in increasing osteopontin gene expression in a population of cells expressing FGFR5 polypeptide when the agonist is contacted with the population of cells. Alternatively, binding agents may be antagonists of FGFR5 polypeptide function that are, for example, effective in decreasing osteopontin gene expression in a population of cells expressing FGFR5 polypeptide when the antagonist is contacted with the population of cells.

Still further embodiments of the present invention provide methods for modulating osteopontin expression in a population of cells. Within certain aspects, these methods comprise the step of contacting the population of cells with one of the compositions recited herein above. Thus, within certain methods, the modulator of FGFR5 gene expression specifically binds to a polynucleotide including: (a) polynucleotides comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9; (b) complements of polynucleotides comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9; (c) reverse sequences of polynucleotides comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9; (d) polynucleotides that encode polypeptides comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15; (e) complements of polynucleotides that encode polypeptides comprising sequences selected from the group consisting of: SEQ ID NO: 5-8 and 13-15; and (f) reverse sequences of polynucleotides that encode polypeptides comprising sequences selected from the group consisting of: SEQ ID NO: 5-8 and 13-15.

Within these methods, the modulator of FGFR5 gene expression is effective in decreasing FGFR5 gene expression when contacted with a population of cells expressing FGFR5 and/or decreasing osteopontin gene expression when contacted with a population of cells expressing FGFR5. Suitable such modulators of FGFR5 gene expression include anti-sense oligonucleotides such as: (a) anti-sense expression vectors; (b) anti-sense oligodeoxyribonucleotides, (c) anti-sense phosphorothioate oligodeoxyribonucleotides, (d) anti-sense oligoribonucleotides, and (e) anti-sense phosphorothioate oligoribonucleotides.

Within other aspects, the present invention provides methods for modulating osteopontin expression in a population of cells the method comprising the step of contacting the population of cells with the composition comprising a binding agent as recited herein above. For example, such methods employ binding agents that specifically bind to polypeptides such as (a) polypeptides encoded by a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9 or a complement thereof; and (b) polypeptides comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15. Binding agents include agonists of FGFR5 polypeptide function wherein binding of the agonists to the population of cells results in an increase in osteopontin expression when the agonist is contacted with the population of cells. Alternatively, binding agents include antagonists of FGFR5 polypeptide function wherein binding of the antagonist to the population of cells results in a decrease in osteopontin expression when the antagonist is contacted with the population of cells.

Still further embodiments of the present invention provide modulators of FGFR5 gene expression in a medicament for the treatment of a disease associated with elevated osteopontin expression. Within certain aspects, the modulator includes: (a) small molecule inhibitors of gene expression, (b) anti-sense oligonucleotides, and (c) small interfering RNA molecules (siRNA or RNAi).

Modulators of FGFR5 gene expression specifically bind to polynucleotides including: (a) polynucleotides comprising sequences selected from the group consisting of SEQ ID NO: 1-4 and 9; (b) complements of polynucleotides comprising sequences selected from the group consisting of SEQ ID NO: 1-4 and 9; (c) reverse sequences of polynucleotides comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9; (d) polynucleotides that encodes a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15; (e) complements of polynucleotides that encodes a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15; and (f) reverse sequences of polynucleotides that encode a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15.

Exemplary diseases associated with elevated osteopontin gene expression that are suitably treated with inventive modulators of FGFR5 gene include cancers, multiple

sclerosis (MS), systemic lupus erythematosus (SLE), diabetes, rheumatoid arthritis (RA), sarcoidosis, tuberculosis, kidney stones, atherosclerosis, vasculitis, nephritis, arthritis, and osteoporosis.

Within other related aspects of the present invention are provided uses of binding agents in medicaments for the treatment of diseases associated with elevated osteopontin expression wherein the binding agents are antagonists of FGFR5 polypeptide function and wherein the binding agents include: (a) small molecules; (b) antibodies or antigen-binding fragments thereof; (c) small chain antibody fragments (scFv); and (d) a camelid heavy chain antibodies (HCAb) or heavy chain variable domains (V_{HH}) thereof.

Exemplary binding agents presented herein specifically bind to polypeptides including: (a) polypeptides encoded by a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9 or a complement thereof; and (b) polypeptides comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15.

Binding agents such as those recited herein may be suitably employed in the treatment of diseases associated with elevated osteopontin expression including cancers, multiple sclerosis (MS), systemic lupus erythematosus (SLE), diabetes, rheumatoid arthritis (RA), sarcoidosis, tuberculosis, kidney stones, atherosclerosis, vasculitis, nephritis, arthritis, and osteoporosis.

Other embodiments of the present invention provide uses of binding agents in medicaments for the treatment of a diseases associated with reduced osteopontin expression wherein the binding agent is an agonist of FGFR5 polypeptide function and wherein the binding agent includes: (a) small molecules; (b) antibodies or antigen-binding fragments thereof; (c) small chain antibody fragments (scFv); (d) camelid heavy chain antibodies (HCAb) or heavy chain variable domains (V_{HH}) thereof; and (e) FGFR5 ligands or FGFR5-binding fragments thereof.

Binding agents suitable for uses in diseases associated with reduced osteopontin expression specifically bind to polypeptides including: (a) polypeptides encoded by polynucleotides comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9 or a complement thereof; and (b) polypeptides comprising a sequence

selected from the group consisting of: SEQ ID NO: 5-8 and 13-15. An exemplary disease associated with reduced osteopontin expression is osteopetrosis.

Other embodiments of the present invention provide methods for the treatment of a disease associated with elevated osteopontin expression that comprise the step of administering to a patient one of the compositions recited herein above. Related aspects of the present invention provide methods for the treatment of cancers, including breast cancer, hepatocellular carcinoma, and colon cancer; methods for the treatment of bone disorders, including osteoporosis and osteopetrosis; and methods for the treatment of FGFR5-associated disorders in a patient. Each of these methods comprise the administration of one or more of the compositions presented herein.

Still further embodiments of the present invention provide methods for inhibiting the expression of osteopontin in populations of cells that comprise reducing the amount of a polypeptide in the cells, wherein the polypeptide comprises an amino acid sequence including: (a) a sequence provided in SEQ ID NO: 5-8 and 13-15; (b) sequences having at least 75% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15; (c) sequences having at least 90% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15; and (d) sequences having at least 95% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15.

Related methods for inhibiting the expression of osteopontin in a population of cells, comprise the step of inhibiting the activity of a polypeptide in the population of cells by administering a composition presented herein wherein said polypeptide comprising an amino acid sequence such as: (a) a sequence provided in SEQ ID NO: 5-8 and 13-15; (b) sequences having at least 75% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15; (c) sequences having at least 90% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15; and (d) sequences having at least 95% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15.

Other methods are provided herein for treating disorders characterized by an elevated level of osteopontin that comprise the step of administering a composition that comprises a binding agent that specifically binds to a polypeptide comprising an amino acid sequence selected from the group consisting of: (a) sequences provided in SEQ ID NO: 5-8 and 13-15; (b) sequences having at least 75% identity to a sequence provided

in SEQ ID NO: 5-8 and 13-15; (c) sequences having at least 90% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15; and (d) sequences having at least 95% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15.

Still further methods are provided herein for treating a disorder characterized by an elevated level of osteopontin, comprising administering a composition presented herein wherein the composition comprises a modulator of FGFR5 gene expression that binds specifically to a polynucleotide comprising a sequence selected from the group consisting of: (a) sequences provided in SEQ ID NO: 1-4 and 9; (b) sequences having at least 75% identity to a sequence provided in SEQ ID NO: 1-4 and 9; (c) sequences having at least 90% identity to a sequence provided in SEQ ID NO: 1-4 and 9; and (d) sequences having at least 95% identity to a sequence provided in SEQ ID NO: 1-4 and 9.

The above-mentioned and additional features of the present invention, together with the manner of obtaining them, will be best understood by reference to the following more detailed description. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

Brief Description of the Drawings

Figure 1 is the amino acid sequence of the murine FGF receptor muFGFR5 β (SEQ ID NO: 6). Several conserved domains were identified that are involved in the dimerization, ligand binding and activity of the receptor. The signal peptide and transmembrane domain are underlined, and the six cysteines conserved among the FGFR family members are in bold and underlined. Four glycosylation sites are double underlined. Three immunoglobulin domains (Ig loops) were identified (Ig loop 1: residues 40-102; Ig loop 2: residues 161-224; Ig loop 3: residues 257-341), as well as two tyrosine kinase phosphorylation sites (residues 198-201, 325-332), a cAMP- and cGMP-dependent protein kinase phosphorylation site (residues 208-215) and four prenyl group binding sites (CAAX boxes). The phosphorylation sites and CAAX boxes are boxed. A heparin binding domain was identified (residues 150-167; boxed and in bold) and this partially overlaps the CAM binding domain (residues 141-160; italics and underlined).

Figure 2A shows the induction of genes under the control of the SRE. NIH-3T3 SRE cells were stimulated with a titration of FGF-2 in the presence of 10 μ g/ml of heparin for 6 hours. Closed circles represent media alone, open squares represent titration of FGF-2. Figure 2B shows the competition analysis of NIH-3T3 SRE cells treated with a standard dose of FGF-2 plus heparin in the presence of increasing concentrations of FGFR2Fc (closed diamonds), FGFR5 β Fc (closed squares), FGFR5 γ Fc (closed triangles) and FGF-2 alone (asterisk). The mean and SD were calculated for both experiments from three separate wells and are represented as fold-induction of the reporter gene relative to control.

Figure 3 illustrates the stimulation of growth of RAW264.10 cells by FGFR5 β and FGFR5 γ . This stimulation was not observed when FGF-2 and FGFR2 were used as controls. This stimulation was also not induced by the growth medium.

Figure 4 illustrates the enhancing proliferative effect of FGFR5 β and FGFR5 γ on PHA-induced PBMC. The enhanced proliferation was not observed when FGFR2 or purified IgG Fc was used.

Figure 5 shows the enhanced proliferation of anti-CD3 stimulated PBMC by FGFR5 β and FGFR5 γ . The enhanced proliferation was not observed when FGFR2 or purified FC was used as stimulants.

Figure 6 demonstrates that FGFR5 β and FGFR5 γ , or the controls FGFR2 or IgG Fc did not stimulate proliferation of PBMC in the absence of PHA.

Figure 7 illustrates the stimulation of PBMC adherence by FGFR5 β and FGFR5 γ but not by FGFR2 or purified IgG Fc.

Figure 8 shows the stimulation of adherent PHA-stimulated PBMC by FGFR5 β and FGFR5 γ but not by purified IgG Fc.

Figure 9 illustrates the stimulation of NK cell adherence by FGFR5 β and FGFR5 γ as measured by the presence of anti-CD56 antibodies, markers of NK cells. The filled histograms represent the adherent PBMC stained with the NK cell marker CD56 and the open histograms represent the same cells stained with the isotype-matched control antibody.

Figure 10 shows the amino acid sequence of human FGFR5 (SEQ ID NO: 8). Several conserved domains were identified that are involved in the dimerization, ligand binding and activity of the receptor. The signal peptide is underlined, and five of the six cysteines conserved among the FGFR family members are in bold and underlined. Three immunoglobulin domains (Ig loops) were identified (Ig loop 1: residues 44-106; Ig loop 2: residues 165-228; Ig loop 3 (partial): residues 261-324), as well as a tyrosine kinase phosphorylation sites (residues 212-219), a cAMP- and cGMP-dependent protein kinase phosphorylation site (residues 202-205) and four prenyl group binding sites (CAAX boxes). The phosphorylation sites and CAAX boxes are boxed. A heparin-binding domain was identified (residues 154-171; boxed and in bold) and this partially overlaps the CAM binding domain (residues 145-164; italics and underlined).

Figure 11A-C are bar graphs depicting upregulation of OPN protein (Fig. 11A), PBMC (Fig. 11B), and adherent PBMC (predominantly monocytes; Fig. 11C) following stimulation with FGFR2, FGFR5, LPS or media alone for 24 hours. Supernatants were collected for cytokine analysis.

Detailed Description of the Invention

In one aspect, the present invention provides polynucleotides isolated from lymph node stromal cells of *fsn* ^{-/-} mice and isolated polypeptides encoded by such polynucleotides, together with human homologues of such polynucleotides and polypeptides.

The term "polynucleotide(s)," as used herein, means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes

all such operable anti-sense fragments. Anti-sense polynucleotides and techniques involving anti-sense polynucleotides are well known in the art and are described, for example, in Robinson-Benion *et al.*, *Methods in Enzymol.* 254: 363-375, 1995 and Kawasaki *et al.*, *Artific. Organs* 20: 836-848, 1996.

5 In specific embodiments, the isolated polynucleotides of the present invention comprise a polynucleotide sequence selected from the group consisting of sequences provided in SEQ ID NO: 1-4 and 9.

Complements of such isolated polynucleotides, reverse complements of such isolated polynucleotides and reverse sequences of such isolated polynucleotides are also
10 provided, together with polynucleotides comprising at least a specified number of contiguous residues (x-mers) of any of the above-mentioned polynucleotides, extended sequences corresponding to any of the above polynucleotides, antisense sequences corresponding to any of the above polynucleotides, and variants of any of the above polynucleotides, as that term is described in this specification.

15 The definitions of the terms "complement", "reverse complement" and "reverse sequence", as used herein, are best illustrated by the following example. For the sequence 5' AGGACC 3', the complement, reverse complement and reverse sequence are as follows:

	complement	3' TCCTGG 5'
20	reverse complement	3' GGTCCT 5'
	reverse sequence	5' CCAGGA 3'.

Preferably, sequences that are complements of a specifically recited polynucleotide sequence are complementary over the entire length of the specific polynucleotide sequence.

25 Some of the polynucleotides of the present invention are "partial" sequences, in that they do not represent a full length gene encoding a full length polypeptide. Such partial sequences may be extended by analyzing and sequencing various DNA libraries using primers and/or probes and well known hybridization and/or PCR techniques. Partial sequences may be extended until an open reading frame encoding a polypeptide, a
30 full length polynucleotide and/or gene capable of expressing a polypeptide, or another useful portion of the genome is identified. Such extended sequences, including full

length polynucleotides and genes, are described as "corresponding to" a sequence identified as one of the sequences of SEQ ID NO: 1-4 and 9, or a variant thereof, or a portion of one of the sequences of SEQ ID NO: 1-4 and 9, or a variant thereof, when the extended polynucleotide comprises an identified sequence or its variant, or an identified
5 contiguous portion (x-mer) of one of the sequences of SEQ ID NO: 1-4 and 9, or a variant thereof. Such extended polynucleotides may have a length of from about 50 to about 4,000 nucleic acids or base pairs, and preferably have a length of less than about 4,000 nucleic acids or base pairs, more preferably yet a length of less than about 3,000 nucleic acids or base pairs, more preferably yet a length of less than about 2,000 nucleic acids or
10 base pairs. Under some circumstances, extended polynucleotides of the present invention may have a length of less than about 1,800 nucleic acids or base pairs, preferably less than about 1,600 nucleic acids or base pairs, more preferably less than about 1,400 nucleic acids or base pairs, more preferably yet less than about 1,200 nucleic acids or base pairs, and most preferably less than about 1,000 nucleic acids or base pairs.

15 Similarly, RNA sequences, reverse sequences, complementary sequences, antisense sequences, and the like, corresponding to the polynucleotides of the present invention, may be routinely ascertained and obtained using the cDNA sequences identified as SEQ ID NO: 1-4.

The polynucleotides identified as SEQ ID NO: 1-4 contain open reading frames
20 ("ORFs") or partial open reading frames encoding polypeptides or functional portions of polypeptides. Open reading frames may be identified using techniques that are well known in the art. These techniques include, for example, analysis for the location of known start and stop codons, most likely reading frame identification based on codon frequencies, etc. Open reading frames and portions of open reading frames may be
25 identified in the polynucleotides of the present invention. Suitable tools and software for ORF analysis are well known in the art and include, for example, GeneWise, available from The Sanger Center, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, United Kingdom; Diogenes, available from Computational Biology Centers, University of Minnesota, Academic Health Center, UMHG Box 43 Minneapolis MN
30 55455; and GRAIL, available from the Informatics Group, Oak Ridge National Laboratories, Oak Ridge, Tennessee TN. Once a partial open reading frame is identified,

the polynucleotide may be extended in the area of the partial open reading frame using techniques that are well known in the art until the polynucleotide for the full open reading frame is identified. Thus, open reading frames encoding polypeptides and/or functional portions of polypeptides may be identified using the polynucleotides of the present invention.

Once open reading frames are identified in the polynucleotides of the present invention, the open reading frames may be isolated and/or synthesized. Expressible genetic constructs comprising the open reading frames and suitable promoters, initiators, terminators, etc., which are well known in the art, may then be constructed. Such genetic constructs may be introduced into a host cell to express the polypeptide encoded by the open reading frame. Suitable host cells may include various prokaryotic and eukaryotic cells, including plant cells, mammalian cells, bacterial cells, algae and the like.

In another aspect, the present invention provides isolated polypeptides encoded, or partially encoded, by the above polynucleotides. The term "polypeptide", as used herein, encompasses amino acid chains of any length including full length proteins, wherein amino acid residues are linked by covalent peptide bonds. Polypeptides of the present invention may be naturally purified products, or may be produced partially or wholly using recombinant techniques. Polypeptides may comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

The term "polypeptide encoded by a polynucleotide" as used herein, includes polypeptides encoded by a nucleotide sequence which includes the partial isolated DNA sequences of the present invention. In specific embodiments, the inventive polypeptides comprise an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO: 5-8, 13-15, and variants of such sequences.

Polypeptides encoded by the polynucleotides of the present invention may be expressed and used in various assays to determine their biological activity. Such polypeptides may be used to raise antibodies, to isolate corresponding interacting proteins

or other compounds, and to quantitatively determine levels of interacting proteins or other compounds.

All of the polynucleotides and polypeptides described herein are isolated and purified, as those terms are commonly used in the art. Preferably, the polypeptides and polynucleotides are at least about 80% pure, more preferably at least about 90% pure, and most preferably at least about 99% pure.

As used herein, the term "variant" comprehends nucleotide or amino acid sequences different from the specifically identified sequences, wherein one or more nucleotides or amino acid residues is deleted, substituted, or added. Variants may be naturally occurring allelic variants, or non-naturally occurring variants. Variant sequences (polynucleotide or polypeptide) preferably exhibit at least 75%, more preferably at least 80%, more preferably yet at least 90%, and most preferably, at least 95% or 98% identity to a sequence of the present invention. The percentage identity may be determined using well known techniques. In one embodiment, the percentage identity is determined by aligning the two sequences to be compared as described below, determining the number of identical residues in the aligned portion, dividing that number by the total number of residues in the inventive (queried) sequence, and multiplying the result by 100.

Polynucleotides and polypeptides having a specified percentage identity to a polynucleotide or polypeptide identified in one of SEQ ID NO: 1-9, 13-15 thus share a high degree of similarity in their primary structure. In addition to a specified percentage identity to a polynucleotide of the present invention, variant polynucleotides and polypeptides preferably have additional structural and/or functional features in common with a polynucleotide of the present invention. Polynucleotides having a specified degree of identity to, or capable of hybridizing to, a polynucleotide of the present invention preferably additionally have at least one of the following features: (1) they contain an open reading frame, or partial open reading frame, encoding a polypeptide, or a functional portion of a polypeptide, having substantially the same functional properties as the polypeptide, or functional portion thereof, encoded by a polynucleotide in a recited SEQ ID NO.; or (2) they contain identifiable domains in common.

Polynucleotide or polypeptide sequences may be aligned, and percentages of identical nucleotides or amino acids in a specified region may be determined against another polynucleotide or polypeptide, using computer algorithms that are publicly available. The BLASTN and FASTA algorithms, set to the default parameters described in the documentation and distributed with the algorithm, may be used for aligning and identifying the similarity of polynucleotide sequences. The alignment and similarity of polypeptide sequences may be examined using the BLASTP algorithm. BLASTX and FASTX algorithms compare nucleotide query sequences translated in all reading frames against polypeptide sequences. The FASTA and FASTX algorithms are described in Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444-2448, 1988; and in Pearson, *Methods in Enzymol.* 183:63-98, 1990. The FASTA software package is available from the University of Virginia by contacting the Assistant Provost for Research, University of Virginia, PO Box 9025, Charlottesville, VA 22906-9025. The BLASTN software is available from the National Center for Biotechnology Information (NCBI), National Library of Medicine, Building 38A, Room 8N805, Bethesda, MD 20894. The BLASTN algorithm Version 2.0.11 [Jan-20-2000] set to the default parameters described in the documentation and distributed with the algorithm, is preferred for use in the determination of polynucleotide variants according to the present invention. The use of the BLAST family of algorithms, including BLASTN, BLASTP and BLASTX, is described in the publication of Altschul *et al.*, "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs," *Nucleic Acids Res.* 25:3389-3402, 1997.

The following running parameters are preferred for determination of alignments and similarities using BLASTN that contribute to the E values and percentage identity for polynucleotides: Unix running command with the following default parameters: blastall -p blastn -d embldb -e 10 -G 0 -E 0 -r 1 -v 30 -b 30 -i queryseq -o results; and parameters are: -p Program Name [String]; -d Database [String]; -e Expectation value (E) [Real]; -G Cost to open a gap (zero invokes default behavior) [Integer]; -E Cost to extend a gap (zero invokes default behavior) [Integer]; -r Reward for a nucleotide match (BLASTN only) [Integer]; -v Number of one-line descriptions (V) [Integer]; -b Number of alignments to show (B) [Integer]; -i Query File [File In]; -o BLAST report Output File [File Out] Optional.

The following running parameters are preferred for determination of alignments and similarities using BLASTP that contribute to the E values and percentage identity of polypeptide sequences: `blastall -p blastp -d swissprot -e 10 -G 0 -E 0 -v 30 -b 30 -i queryseq -o results`; the parameters are: -p Program Name [String]; -d Database [String];
 5 -e Expectation value (E) [Real]; -G Cost to open a gap (zero invokes default behavior) [Integer]; -E Cost to extend a gap (zero invokes default behavior) [Integer]; -v Number of one-line descriptions (v) [Integer]; -b Number of alignments to show (b) [Integer]; -I Query File [File In]; -o BLAST report Output File [File Out] Optional.

The "hits" to one or more database sequences by a queried sequence produced by
 10 BLASTN, BLASTP, FASTA, or a similar algorithm, align and identify similar portions of sequences. The hits are arranged in order of the degree of similarity and the length of sequence overlap. Hits to a database sequence generally represent an overlap over only a fraction of the sequence length of the queried sequence.

As noted above, the percentage identity of a polynucleotide or polypeptide
 15 sequence is determined by aligning polynucleotide and polypeptide sequences using appropriate algorithms, such as BLASTN or BLASTP, respectively, set to default parameters; identifying the number of identical nucleic or amino acids over the aligned portions; dividing the number of identical nucleic or amino acids by the total number of nucleic or amino acids of the polynucleotide or polypeptide of the present invention; and
 20 then multiplying by 100 to determine the percentage identity. By way of example, a queried polynucleotide having 220 nucleic acids has a hit to a polynucleotide sequence in the EMBL database having 520 nucleic acids over a stretch of 23 nucleotides in the alignment produced by the BLASTN algorithm using the default parameters. The 23-nucleotide hit includes 21 identical nucleotides, one gap and one different nucleotide.
 25 The percentage identity of the queried polynucleotide to the hit in the EMBL database is thus $21/220$ times 100, or 9.5%. The percentage identity of polypeptide sequences may be determined in a similar fashion.

The BLASTN and BLASTX algorithms also produce "Expect" values for polynucleotide and polypeptide alignments. The Expect value (E) indicates the number of
 30 hits one can "expect" to see over a certain number of contiguous sequences by chance when searching a database of a certain size. The Expect value is used as a significance

threshold for determining whether the hit to a database indicates true similarity. For example, an E value of 0.1 assigned to a polynucleotide hit is interpreted as meaning that in a database of the size of the EMBL database, one might expect to see 0.1 matches over the aligned portion of the sequence with a similar score simply by chance. By this criterion, the aligned and matched portions of the sequences then have a probability of 90% of being related. For sequences having an E value of 0.01 or less over aligned and matched portions, the probability of finding a match by chance in the EMBL database is 1% or less using the BLASTN algorithm. E values for polypeptide sequences may be determined in a similar fashion using various polypeptide databases, such as the SwissProt database.

According to one embodiment, "variant" polynucleotides and polypeptides, with reference to each of the polynucleotides and polypeptides of the present invention, preferably comprise sequences having the same number or fewer nucleotides or amino acids than each of the polynucleotides or polypeptides of the present invention and producing an E value of 0.01 or less when compared to the polynucleotide or polypeptide of the present invention. That is, a variant polynucleotide or polypeptide is any sequence that has at least a 99% probability of being related to the polynucleotide or polypeptide of the present invention, measured as having an E value of 0.01 or less using the BLASTN or BLASTX algorithms set at the default parameters. According to a preferred embodiment, a variant polynucleotide is a sequence having the same number or fewer nucleic acids than a polynucleotide of the present invention that has at least a 99% probability of being related to the polynucleotide of the present invention, measured as having an E value of 0.01 or less using the BLASTN algorithm set at the default parameters. Similarly, according to a preferred embodiment, a variant polypeptide is a sequence having the same number or fewer amino acids than a polypeptide of the present invention that has at least a 99% probability of being related as the polypeptide of the present invention, measured as having an E value of 0.01 or less using the BLASTP algorithm set at the default parameters.

In an alternative embodiment, variant polynucleotides are sequences that hybridize to a polynucleotide of the present invention under stringent conditions. Stringent hybridization conditions for determining complementarity include salt

conditions of less than about 1 M, more usually less than about 500 mM, and preferably less than about 200 mM. Hybridization temperatures can be as low as 5°C, but are generally greater than about 22°C, more preferably greater than about 30°C, and most preferably greater than about 37°C. Longer DNA fragments may require higher hybridization temperatures for specific hybridization. Since the stringency of hybridization may be affected by other factors such as probe composition, presence of organic solvents, and extent of base mismatching, the combination of parameters is more important than the absolute measure of any one alone. An example of "stringent conditions" is prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65°C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65°C and two washes of 30 minutes each in 0.2X SSC, 0.1% SDS at 65°C.

The present invention also encompasses polynucleotides that differ from the disclosed sequences but that, as a consequence of the discrepancy of the genetic code, encode a polypeptide having similar enzymatic activity to a polypeptide encoded by a polynucleotide of the present invention. Thus, polynucleotides comprising sequences that differ from the polynucleotide sequences recited in SEQ ID NO: 1-4, or complements, reverse sequences, or reverse complements of those sequences, as a result of conservative substitutions are contemplated by and encompassed within the present invention. Additionally, polynucleotides comprising sequences that differ from the polynucleotide sequences recited in SEQ ID NO: 1-4, or complements, reverse complements or reverse sequences thereof, as a result of deletions and/or insertions totaling less than 10% of the total sequence length are also contemplated by and encompassed within the present invention. Similarly, polypeptides comprising sequences that differ from the polypeptide sequences recited in SEQ ID NO: 5-8 and 13-15 as a result of amino acid substitutions, insertions, and/or deletions totaling less than 10% of the total sequence length are contemplated by and encompassed within the present invention, provided the variant polypeptide has functional properties which are substantially the same as, or substantially similar to those of a polypeptide comprising a sequence of SEQ ID NO: 5-8 and 13-15.

Polynucleotides of the present invention also comprehend polynucleotides comprising at least a specified number of contiguous residues (x-mers) of any of the

polynucleotides identified as SEQ ID NO: 1-4 and 9, complements, reverse sequences, and reverse complements of such sequences, and their variants. Similarly, polypeptides of the present invention comprehend polypeptides comprising at least a specified number of contiguous residues (x-mers) of any of the polypeptides identified as SEQ ID NO: 5-8, 13-15, and their variants. As used herein, the term "x-mer," with reference to a specific value of "x," refers to a sequence comprising at least a specified number ("x") of contiguous residues of any of the polynucleotides identified as SEQ ID NO: 1-4 and 9, or the polypeptides identified as SEQ ID NO: 5-8 and 13-15. According to preferred embodiments, the value of x is preferably at least 20, more preferably at least 40, more preferably yet at least 60, and most preferably at least 80. Thus, polynucleotides and polypeptides of the present invention comprise a 20-mer, a 40-mer, a 60-mer, an 80-mer, a 100-mer, a 120-mer, a 150-mer, a 180-mer, a 220-mer, a 250-mer, a 300-mer, 400-mer, 500-mer or 600-mer of a polynucleotide or polypeptide identified as SEQ ID NO: 1-9, 13-15, and variants thereof.

The inventive polynucleotides may be isolated by high throughput sequencing of cDNA libraries prepared from lymph node stromal cells of *fsn* ^{-/-} mice as described below in Example 1. Alternatively, oligonucleotide probes based on the sequences provided in SEQ ID NO: 1-4 and 9 can be synthesized and used to identify positive clones in either cDNA or genomic DNA libraries from lymph node stromal cells of *fsn* ^{-/-} mice by means of hybridization or polymerase chain reaction (PCR) techniques. Probes can be shorter than the sequences provided herein but should be at least about 10, preferably at least about 15 and most preferably at least about 20 nucleotides in length. Hybridization and PCR techniques suitable for use with such oligonucleotide probes are well known in the art (see, for example, Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol.*, 51:263, 1987; Erlich ed., *PCR Technology*, Stockton Press, NY, 1989; Sambrook *et al.*, *Molecular cloning – a laboratory manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). Positive clones may be analyzed by restriction enzyme digestion, DNA sequencing or the like.

The polynucleotides of the present invention may alternatively be synthesized using techniques that are well known in the art. The polynucleotides may be synthesized, for example, using automated oligonucleotide synthesizers (e.g., Beckman Oligo 1000M

DNA Synthesizer) to obtain polynucleotide segments of up to 50 or more nucleic acids. A plurality of such polynucleotide segments may then be ligated using standard DNA manipulation techniques that are well known in the art of molecular biology. One conventional and exemplary polynucleotide synthesis technique involves synthesis of a
5 single stranded polynucleotide segment having, for example, 80 nucleic acids, and hybridizing that segment to a synthesized complementary 85 nucleic acid segment to produce a 5 nucleotide overhang. The next segment may then be synthesized in a similar fashion, with a 5 nucleotide overhang on the opposite strand. The "sticky" ends ensure proper ligation when the two portions are hybridized. In this way, a complete
10 polynucleotide of the present invention may be synthesized entirely *in vitro*.

Polypeptides of the present invention may be produced recombinantly by inserting a DNA sequence that encodes the polypeptide into an expression vector and expressing the polypeptide in an appropriate host. Any of a variety of expression vectors known to those of ordinary skill in the art may be employed. Expression may be
15 achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, insect, yeast or a mammalian cell line such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring
20 polypeptides, portions of naturally occurring polypeptides, or other variants thereof.

In a related aspect, polypeptides are provided that comprise at least a functional portion of a polypeptide having an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO: 5-8, 13-15, and variants thereof. As used herein, the "functional portion" of a polypeptide is that portion which contains the
25 active site essential for affecting the function of the polypeptide, for example, the portion of the molecule that is capable of binding one or more reactants. The active site may be made up of separate portions present on one or more polypeptide chains and will generally exhibit high binding affinity. Such functional portions generally comprise at least about 5 amino acid residues, more preferably at least about 10, and most preferably
30 at least about 20 amino acid residues. Functional portions of the inventive polypeptides may be identified by first preparing fragments of the polypeptide, by either chemical or

enzymatic digestion of the polypeptide or mutation analysis of the polynucleotide that encodes for the polypeptide, and subsequently expressing the resultant mutant polypeptides. The polypeptide fragments or mutant polypeptides are then tested to determine which portions retain the biological activity of the full-length polypeptide.

5 Portions and other variants of the inventive polypeptides may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase
10 techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain (Merrifield, *J. Am. Chem. Soc.* 85:2149-2154, 1963). Equipment for automated synthesis of polypeptides is available from suppliers such as Perkin Elmer/Applied BioSystems, Inc. (Foster City, CA), and may be operated according to the manufacturer's instructions. Variants of a native
15 polypeptide may be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis (see, for example, Kunkel, *Proc. Natl. Acad. Sci. USA* 82:488-492, 1985). Sections of DNA sequence may also be removed using standard techniques to permit preparation of truncated polypeptides.

The present invention also provides fusion proteins comprising a first and a
20 second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known polypeptide, together with variants of such fusion proteins. The fusion proteins of the present invention may include a linker peptide between the first and second polypeptides.

A polynucleotide encoding a fusion protein of the present invention is constructed
25 using known recombinant DNA techniques to assemble separate polynucleotides encoding the first and second polypeptides into an appropriate expression vector. The 3' end of a polynucleotide encoding the first polypeptide is ligated, with or without a peptide linker, to the 5' end of a DNA sequence polynucleotide encoding the second polypeptide so that the reading frames of the sequences are in phase to permit mRNA
30 translation of the two polynucleotides into a single fusion protein that retains the biological activity of both the first and the second polypeptides.

A peptide linker sequence may be employed to separate the first and the second polypeptides by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., *Gene* 40:39-46, 1985; Murphy et al., *Proc. Natl. Acad. Sci. USA* 83:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may be from 1 to about 50 amino acids in length. Peptide linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The ligated polynucleotides encoding the fusion proteins are cloned into suitable expression systems using techniques known to those of ordinary skill in the art.

The polynucleotide sequences of the present invention encode polypeptides that have important role(s) in growth and development of the immune system, and in responses of the immune system to tissue injury and inflammation as well as other disease states. Some of the polynucleotides contain sequences that code for signal sequences, or transmembrane domains, which identify the protein products as secreted molecules or receptors. The polypeptides of SEQ ID NO: 5-8 have more than 25% identity to members of the fibroblast growth factor (FGF) receptor family of proteins. The inventive polypeptides have important roles in processes such as: modulation of immune responses; differentiation of precursor immune cells into specialized cell types; cell migration; cell proliferation and cell-cell interaction. The polypeptides are important in the defence of the body against infectious agents, and thus important in maintaining a disease-free environment. These polypeptides act as modulators of skin cells, especially

since immune cells infiltrate skin during tissue insult, causing growth and differentiation of skin cells. In addition, these polypeptides are immunologically active, making them important therapeutic targets in a large range of disease states.

In one aspect, the present invention provides methods for using one or more of the inventive polypeptides or polynucleotides to treat a disorder in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human.

In this aspect, the polypeptide or polynucleotide is generally present within a composition, such as a pharmaceutical or immunogenic composition. Pharmaceutical compositions may comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. Immunogenic compositions may comprise one or more of the above polypeptides and an immunostimulant, such as an adjuvant or a liposome, into which the polypeptide is incorporated.

Alternatively, a composition of the present invention may contain DNA encoding one or more polypeptides described above, such that the polypeptide is generated *in situ*. In such compositions, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, and bacterial and viral expression systems. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminator signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus Calmette-Guerin*) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other poxvirus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic, or defective, replication competent virus. Techniques for incorporating DNA into such expression systems are well known in the art. The DNA may also be "naked," as described, for example, in Ulmer *et al.*, *Science* 259:1745-1749, 1993 and reviewed by Cohen, *Science* 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

Routes and frequency of administration, as well as dosage, vary from individual to individual. In general, the inventive compositions may be administered by injection (e.g., intradermal, intramuscular, intravenous or subcutaneous), intranasally (e.g., by aspiration) or orally. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg per kg of host, and preferably from about 100 pg to about 1 µg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 ml to about 2 ml.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a lipid, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Any of a variety of adjuvants may be employed in the immunogenic compositions of the present invention to non-specifically enhance the immune response. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a non-specific stimulator of immune responses, such as lipid A, *Bordetella pertussis* or *M. tuberculosis*. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Freund's Complete Adjuvant (Difco Laboratories, Detroit, MI), and Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ). Other suitable adjuvants include alum, biodegradable microspheres, monophosphoryl lipid A and Quil A.

The polynucleotides of the present invention may also be used as markers for tissue, as chromosome markers or tags, in the identification of genetic disorders, and for the design of oligonucleotides for examination of expression patterns using techniques well known in the art, such as the microarray technology available from Affymetrix

(Santa Clara, CA). Partial polynucleotide sequences disclosed herein may be employed to obtain full length genes by, for example, screening of DNA expression libraries, and to isolate homologous DNA sequences from other species using hybridization probes or PCR primers based on the inventive sequences.

5 The isolated polynucleotides of the present invention also have utility in genome mapping, in physical mapping, and in positional cloning of genes. As detailed below, the polynucleotide sequences identified as SEQ ID NO: 1-4, and their variants, may be used to design oligonucleotide probes and primers. Oligonucleotide probes designed using the polynucleotides of the present invention may be used to detect the presence and examine
10 the expression patterns of genes in any organism having sufficiently similar DNA and RNA sequences in their cells using techniques that are well known in the art, such as slot blot DNA hybridization techniques. Oligonucleotide primers designed using the polynucleotides of the present invention may be used for PCR amplifications. Oligonucleotide probes and primers designed using the polynucleotides of the present
15 invention may also be used in connection with various microarray technologies, including the microarray technology of Affymetrix (Santa Clara, CA).

As used herein, the term "oligonucleotide" refers to a relatively short segment of a polynucleotide sequence, generally comprising between 6 and 60 nucleotides, and comprehends both probes for use in hybridization assays and primers for use in the
20 amplification of DNA by polymerase chain reaction. An oligonucleotide probe or primer is described as "corresponding to" a polynucleotide of the present invention, including one of the sequences set out as SEQ ID NO: 1-4 and 9, or a variant thereof, if the oligonucleotide probe or primer, or its complement, is contained within one of the sequences set out as SEQ ID NO: 1-4 and 9, or a variant of one of the specified
25 sequences. Oligonucleotide probes and primers of the present invention are substantially complementary to a polynucleotide disclosed herein.

Two single stranded sequences are said to be substantially complementary when the nucleotides of one strand, optimally aligned and compared, with the appropriate nucleotide insertions and/or deletions, pair with at least 80%, preferably at least 90% to
30 95% and more preferably at least 98% to 100% of the nucleotides of the other strand. Alternatively, substantial complementarity exists when a first DNA strand will selectively

hybridize to a second DNA strand under stringent hybridization conditions. Stringent hybridization conditions for determining complementarity include salt conditions of less than about 1 M, more usually less than about 500 mM, and preferably less than about 200 mM. Hybridization temperatures can be as low as 5°C, but are generally greater than about 22°C, more preferably greater than about 30°C, and most preferably greater than about 37°C. Longer DNA fragments may require higher hybridization temperatures for specific hybridization. Since the stringency of hybridization may be affected by other factors such as probe composition, presence of organic solvents and extent of base mismatching, the combination of parameters is more important than the absolute measure of any one alone.

In specific embodiments, the oligonucleotide probes and/or primers comprise at least about 6 contiguous residues, more preferably at least about 10 contiguous residues, and most preferably at least about 20 contiguous residues complementary to a polynucleotide sequence of the present invention. Probes and primers of the present invention may be from about 8 to 100 base pairs in length or, preferably from about 10 to 50 base pairs in length or, more preferably from about 15 to 40 base pairs in length. The probes can be easily selected using procedures well known in the art, taking into account DNA-DNA hybridization stringencies, annealing and melting temperatures, and potential for formation of loops and other factors, which are well known in the art. Tools and software suitable for designing probes and PCR primers are well known in the art and include the software program available from Premier Biosoft International, 3786 Corina Way, Palo Alto, CA 94303-4504. Preferred techniques for designing PCR primers are also disclosed in Dieffenbach, CW and Dyksler, GS. *PCR Primer: a laboratory manual*, CSHL Press: Cold Spring Harbor, NY, 1995.

A plurality of oligonucleotide probes or primers corresponding to a polynucleotide of the present invention may be provided in a kit form. Such kits generally comprise multiple DNA or oligonucleotide probes or primers, each probe or primer being specific for a polynucleotide sequence. Kits of the present invention may comprise one or more probes or primers corresponding to a polynucleotide of the present invention, including a polynucleotide sequence identified in SEQ ID NO: 1-4 and 9.

In one embodiment useful for high-throughput assays, the oligonucleotide probe kits of the present invention comprise multiple probes in an array format, wherein each probe is immobilized at a predefined, spatially addressable, location on the surface of a solid substrate. Array formats which may be usefully employed in the present invention are disclosed, for example, in U.S. Patents No. 5,412,087 and 5,545,451, and PCT
5 Publication No. WO 95/00450, the disclosures of which are hereby incorporated by reference.

The polynucleotides of the present invention may also be used to tag or identify an organism or reproductive material therefrom. Such tagging may be accomplished, for
10 example, by stably introducing a non-disruptive non-functional heterologous polynucleotide identifier into an organism, the polynucleotide comprising one of the polynucleotides of the present invention.

The polypeptides provided by the present invention may additionally be used in assays to determine biological activity, to raise antibodies, to isolate corresponding
15 ligands or receptors, in assays to quantify levels of protein or cognate corresponding ligand or receptor, as anti-inflammatory agents, and in compositions for the treatment of diseases of the immune system.

The present invention further provides methods and compositions for modulating the levels and/or inhibiting the activity of an inventive polypeptide or polynucleotide. As
20 used herein, the term "modulate" or "modulating" is meant to include an increase or a decrease in polynucleotide expression and/or an increase or a decrease in polypeptide function. Thus, as the term "modulate" is used within the context of polypeptide function, a "modulator" broadly encompasses both "agonists" of protein function and "antagonists" of protein function wherein the term "agonists" refers to, for example,
25 modulator molecules, compounds, and/or compositions that increase polypeptide function whereas the term "antagonist" refers to modulators that decrease polypeptide function.

Methods employing modulators of the present invention include administering a molecule, compound and/or composition selected from the group consisting of: antibodies, antigen-binding fragments thereof, small chain antibody variable domain
30 fragments (scFv), and/or camelid heavy chain antibody (HCAb) or heavy chain variable domain thereof (V_{HH}) that specifically bind to a polypeptide of the present invention;

soluble ligands that bind to an inventive polypeptide; small molecule inhibitors of the inventive polypeptides and/or polynucleotides; anti-sense oligonucleotides to the inventive polynucleotides; small interfering RNA molecules (siRNA or RNAi) that are specific for a polynucleotide or polypeptide of the present invention; and engineered
5 soluble polypeptide molecules that bind a ligand of an inventive polypeptide but do not stimulate signaling.

Modulating the activity of a polypeptide described herein may be accomplished by reducing or inhibiting expression of the polypeptides, which can be achieved by interfering with transcription and/or translation of the corresponding polynucleotide.
10 Polypeptide expression may be inhibited, for example, by introducing anti-sense expression vectors; by introducing anti-sense oligodeoxyribonucleotides, anti-sense phosphorothioate oligodeoxyribonucleotides, anti-sense oligoribonucleotides or anti-sense phosphorothioate oligoribonucleotides; or by other means well known in the art. All such anti-sense polynucleotides are referred to collectively herein as "anti-sense
15 oligonucleotides".

The anti-sense oligonucleotides disclosed herein are sufficiently complementary to the polynucleotide encoding the inventive polypeptide to bind specifically to the polynucleotide. The sequence of an anti-sense oligonucleotide need not be 100% complementary to that of the polynucleotide in order for the anti-sense oligonucleotide to
20 be effective in the inventive methods. Rather an anti-sense oligonucleotide is sufficiently complementary when binding of the anti-sense oligonucleotide to the polynucleotide interferes with the normal function of the polynucleotide to cause a loss of utility, and when non-specific binding of the oligonucleotide to other, non-target, sequences is avoided. The present invention thus encompasses polynucleotides in an anti-sense
25 orientation that inhibit translation of the inventive polypeptides. The design of appropriate anti-sense oligonucleotides is well known in the art. Oligonucleotides that are complementary to the 5' end of the message, for example the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, oligonucleotides complementary to either the 5'- or 3'-non-
30 translated, non-coding, regions of the targeted polynucleotide can be used.

Cell permeation and activity of anti-sense oligonucleotides can be enhanced by appropriate chemical modifications, such as the use of phenoxazine-substituted C-5 propynyl uracil oligonucleotides (Flanagan *et al.*, *Nat. Biotechnol.* 17:48-52 (1999)) or 2'-O-(2-methoxy) ethyl (2'-MOE)-oligonucleotides (Zhang *et al.*, *Nat. Biotechnol.* 18:862-867 (2000)). The use of techniques involving anti-sense oligonucleotides is well known in the art and is described, for example, in Robinson-Benion *et al.*, *Methods in Enzymol.* 254:363-375 (1995) and Kawasaki *et al.*, *Artific. Organs* 20:836-848 (1996).

Expression of a polypeptide of the present invention may also be specifically suppressed by methods such as RNA interference (RNAi). A review of this technique is found in *Science*, 288:1370-1372, 2000. Briefly, traditional methods of gene suppression, employing anti-sense RNA or DNA, operate by binding to the reverse sequence of a gene of interest such that binding interferes with subsequent cellular processes and therefore blocks synthesis of the corresponding protein. RNAi also operates on a post-translational level and is sequence specific, but suppresses gene expression far more efficiently. Exemplary methods for controlling or modifying gene expression are provided in WO 99/49029, WO 99/53050 and WO01/75164, the disclosures of which are hereby incorporated by reference. In these methods, post-transcriptional gene silencing is brought about by a sequence-specific RNA degradation process which results in the rapid degradation of transcripts of sequence-related genes. Studies have shown that double-stranded RNA may act as a mediator of sequence-specific gene silencing (see, for example, Montgomery and Fire, *Trends in Genetics*, 14:255-258, 1998). Gene constructs that produce transcripts with self-complementary regions are particularly efficient at gene silencing.

It has been demonstrated that one or more ribonucleases specifically bind to and cleave double-stranded RNA into short fragments. The ribonuclease(s) remains associated with these fragments, which in turn specifically bind to complementary mRNA, i.e. specifically bind to the transcribed mRNA strand for the gene of interest. The mRNA for the gene is also degraded by the ribonuclease(s) into short fragments, thereby obviating translation and expression of the gene. Additionally, an RNA-polymerase may act to facilitate the synthesis of numerous copies of the short fragments, which exponentially increases the efficiency of the system. A unique feature of RNAi is

that silencing is not limited to the cells where it is initiated. The gene-silencing effects may be disseminated to other parts of an organism.

The polynucleotides of the present invention may thus be employed to generate gene silencing constructs and/or gene-specific self-complementary, double-stranded RNA sequences that can be delivered by conventional art-known methods. A gene construct may be employed to express the self-complementary RNA sequences. Alternatively, cells are contacted with gene-specific double-stranded RNA molecules, such that the RNA molecules are internalized into the cell cytoplasm to exert a gene silencing effect. The double-stranded RNA must have sufficient homology to the targeted gene to mediate RNAi without affecting expression of non-target genes. The double-stranded DNA is at least 20 nucleotides in length, and is preferably 21-23 nucleotides in length. Preferably, the double-stranded RNA corresponds specifically to a polynucleotide of the present invention. The use of small interfering RNA (siRNA) molecules of 21-23 nucleotides in length to suppress gene expression in mammalian cells is described in WO 01/75164. Tools for designing optimal inhibitory siRNAs include that available from DNAengine Inc. (Seattle, WA).

One RNAi technique employs genetic constructs within which sense and anti-sense sequences are placed in regions flanking an intron sequence in proper splicing orientation with donor and acceptor splicing sites. Alternatively, spacer sequences of various lengths may be employed to separate self-complementary regions of sequence in the construct. During processing of the gene construct transcript, intron sequences are spliced-out, allowing sense and anti-sense sequences, as well as splice junction sequences, to bind forming double-stranded RNA. Select ribonucleases then bind to and cleave the double-stranded RNA, thereby initiating the cascade of events leading to degradation of specific mRNA gene sequences, and silencing specific genes.

As used herein, the phrase "contacting a population of cells with a genetic construct, anti-sense oligonucleotide or RNA molecule" includes any means of introducing a nucleic acid molecule into any portion of one or more cells by any method compatible with cell viability and known to those of ordinary skill in the art. The cell or cells may be contacted *in vivo*, *ex vivo*, *in vitro*, or any combination thereof.

For *in vivo* uses, a genetic construct, anti-sense oligonucleotide or RNA molecule may be administered by various art-recognized procedures. See, e.g., Rolland, *Crit. Rev. Therap. Drug Carrier Systems* 15:143-198 (1998), and cited references. Both viral and non-viral delivery methods have been used for gene therapy. Useful viral vectors include, for example, adenovirus, adeno-associated virus (AAV), retrovirus, vaccinia virus and avian poxvirus. Improvements have been made in the efficiency of targeting genes to tumor cells with adenoviral vectors, for example, by coupling adenovirus to DNA-polylysine complexes and by strategies that exploit receptor-mediated endocytosis for selective targeting. See, e.g., Curiel et al., *Hum. Gene Ther.*, 3:147-154 (1992); and Cristiano and Curiel, *Cancer Gene Ther.* 3:49-57 (1996). Non-viral methods for delivering polynucleotides are reviewed in Chang & Seymour, (Eds) *Curr. Opin. Mol. Ther.*, vol. 2 (2000). These methods include contacting cells with naked DNA, cationic liposomes, or polyplexes of polynucleotides with cationic polymers and dendrimers for systemic administration (Chang & Seymour, *Ibid.*). Liposomes can be modified by incorporation of ligands that recognize cell-surface receptors and allow targeting to specific receptors for uptake by receptor-mediated endocytosis. See, for example, Xu et al., *Mol. Genet. Metab.*, 64:193-197 (1998); and Xu et al., *Hum. Gene Ther.*, 10:2941-2952 (1999).

Tumor-targeting bacteria, such as *Salmonella*, are potentially useful for delivering genes to tumors following systemic administration (Low et al., *Nat. Biotechnol.* 17:37-41 (1999)). Bacteria can be engineered *ex vivo* to penetrate and to deliver DNA with high efficiency into mammalian epithelial cells *in vivo* and *in vitro*. See, e.g., Grillot-Courvalin et al., *Nat. Biotechnol.* 16:862-866 (1998). Degradation-stabilized oligonucleotides may be encapsulated into liposomes and delivered to patients by injection either intravenously or directly into a target site. Alternatively, retroviral or adenoviral vectors, or naked DNA expressing anti-sense RNA for the inventive polypeptides, may be delivered into patient's cells *in vitro* or directly into patients *in vivo* by appropriate routes. Suitable techniques for use in such methods are well known in the art.

The present invention further provides binding agents, such as antibodies and antigen-binding fragments thereof, small chain antibody variable domain fragments (scFv), and/or camelid heavy chain antibody (HCAb) or heavy chain variable domain

thereof (V_{HH}) which specifically bind to a polypeptide disclosed herein, or to a portion or variant thereof. A binding agent is said to "specifically bind" to an inventive polypeptide if it reacts at a detectable level with the polypeptide, and does not react detectably with unrelated polypeptides under similar conditions. Any agent that satisfies this requirement
5 may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule, or a polypeptide. In a preferred embodiment, a binding agent is an antibody, an antigen-binding fragment thereof, small chain antibody variable domain fragments (scFv), and/or camelid heavy chain antibody (HCAb) or heavy chain variable domain thereof (V_{HH}). The ability of a binding agent to
10 specifically bind to a polypeptide can be determined, for example, in an ELISA assay using techniques well known in the art.

An "antigen-binding site," or "antigen-binding fragment" of an antibody refers to the part of the antibody that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy
15 ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy and light chains are referred to as "hypervariable regions" which are interposed between more conserved flanking stretches known as "framework regions," or "FRs". Thus the term "FR" refers to amino acid sequences which are naturally found between and adjacent to hypervariable regions in immunoglobulins. In an antibody molecule, the
20 three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as "complementarity-determining regions," or "CDRs."

25 Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell
30 hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the inventive polypeptide is initially injected into any of a

wide variety of mammals (e.g., mice, rats, rabbits, sheep or goats). The polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the inventive polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

10 Monoclonal antibodies specific for an inventive polypeptide may be prepared using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. These methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity. Such cell lines may be produced from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques well known in the art may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

25 Monoclonal antibodies may then be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in,

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for example, an affinity chromatography step.

A number of molecules are known in the art that comprise antigen-binding sites capable of exhibiting the binding properties of an antibody molecule. For example, the proteolytic enzyme papain preferentially cleaves IgG molecules to yield several fragments, two of which (the "F(ab)" fragments) each comprise a covalent heterodimer that includes an intact antigen-binding site. The enzyme pepsin is able to cleave IgG molecules to provide several fragments, including the "F(ab')₂" fragment, which comprises both antigen-binding sites. An "Fv" fragment can be produced by preferential proteolytic cleavage of an IgM, IgG or IgA immunoglobulin molecule, but are more commonly derived using recombinant techniques known in the art. The Fv fragment includes a non-covalent V_H::V_L heterodimer including an antigen-binding site which retains much of the antigen recognition and binding capabilities of the native antibody molecule (Inbar *et al.* *Proc. Nat. Acad. Sci. USA* 69:2659-2662 (1972); Hochman *et al.* *Biochem* 15:2706-2710 (1976); and Ehrlich *et al.* *Biochem* 19:4091-4096 (1980)).

The present invention further encompasses humanized antibodies that specifically bind to an inventive polypeptide. A number of humanized antibody molecules comprising an antigen-binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent V regions and their associated CDRs fused to human constant domains (Winter *et al.* *Nature* 349:293-299 (1991); Lobuglio *et al.* *Proc. Nat. Acad. Sci. USA* 86:4220-4224 (1989); Shaw *et al.* *J Immunol.* 138:4534-4538 (1987); and Brown *et al.* *Cancer Res.* 47:3577-3583 (1987)); rodent CDRs grafted into a human supporting FR prior to fusion with an appropriate human antibody constant domain (Riechmann *et al.* *Nature* 332:323-327 (1988); Verhoeven *et al.* *Science* 239:1534-1536 (1988); and Jones *et al.* *Nature* 321:522-525 (1986)); and rodent CDRs supported by recombinantly veneered rodent FRs (European Patent Publication No. 519,596, published Dec. 23, 1992). These "humanized" molecules are designed to minimize unwanted immunological responses towards rodent antihuman antibody molecules which limit the duration and effectiveness of therapeutic applications of those moieties in human recipients.

Equally suited to the practice of the present invention are single-chain antibodies fragments, including scFv and *Camelidae* heavy chain antibodies (HCAb) that

specifically bind to one of the FGFR5 polypeptides presented as SEQ ID NOs: 5-8, 13-15 as well as variants of any of these polypeptides. ScFv presented herein comprise an antibody heavy chain variable region (V_H) operably linked to an antibody light chain variable region (V_L) wherein the heavy chain variable region and the light chain variable region together or individually form a binding site for specifically binding an FGFR5 polypeptide presented herein. ScFv may comprise a V_H region at the amino-terminal end and a V_L region at the carboxy-terminal end. Equally suitable are scFv that comprise a V_L region at the amino-terminal end and a V_H region at the carboxy-terminal end.

ScFv disclosed herein may, optionally, further comprise a polypeptide linker operably linked between the heavy chain variable region and the light chain variable region. Polypeptide linkers of the present invention generally comprise between 1 and 50 amino acids. More preferred are polypeptide linkers of at least 2 amino acids. Within other embodiments, however, polypeptide linkers are preferably between 3 and 12 amino acids. An exemplary linker peptide for incorporating between scFv heavy and light chains comprises the 5 amino acid sequence Gly-Gly-Gly-Gly-Ser. Alternative exemplary linker peptides comprise one or more tandem repeats of the sequence Gly-Gly-Gly-Gly-Ser to create linkers comprising, for example, the sequences Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser, Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser, and Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser.

Other embodiments of the present invention provide *Camelidae* heavy chain antibodies (HCAb) that specifically bind to polypeptides presented as SEQ ID NOs: 5-8, 13-15 and variants thereof. These heavy chain antibodies are a class of IgG that are devoid of light chains that are produced by animals of the genus *Camelidae* (including camels, dromedaries, and llamas). Hamers-Casterman *et al.*, *Nature* 363:446-448 (1993). HCABs have a molecular weight of ~95 kDa instead of the ~160 kDa for conventional IgG antibodies. Their binding domains consist only of the heavy-chain variable domains, referred to as V_{HH} to distinguish them from conventional V_H s. Muyldermans *et al.*, *J. Mol. Recognit.* 12:131-140 (1999). Since the first constant domain (C_H1) is absent (spliced out during mRNA processing due to loss of a splice consensus signal), the variable domain (V_{HH}) is immediately followed by the hinge region, the C_H2 and the C_H3

domains. Nguyen *et al.*, *Mol. Immunol.* 36:515-524 (1999); Woolven *et al.*, *Immunogenetics* 50:98-101 (1999). Although the HCABs are devoid of light chains, they have an authentic antigen-binding repertoire. The current knowledge about the genetic generation mechanism of HCABs is reviewed by Nguyen *et al.* *Adv. Immunol* 79:261-296 (2001) and Nguyen *et al.*, *Immunogenetics* 54:39-47 (2002). Similarly, sharks, including the nurse shark, display antigen receptor-containing single monomeric V-domains. Irving *et al.*, *J. Immunol. Methods* 248:31-45 (2001); Roux *et al.*, *Proc. Natl. Acad. Sci. USA* 95:11804 (1998).

V_{HHS} comprise the smallest available intact antigen-binding fragment (~15 kDa, 118-136 residues). The affinities of V_{HHS} are typically in the nanomolar range and comparable with those of Fab and scFv fragments. In addition, V_{HHS} are highly soluble and more stable than the corresponding derivatives of scFv and Fab fragments. V_{HHS} carry amino acid substitutions that make them more hydrophilic and prevent the prolonged interaction with BiP (Immunoglobulin heavy-chain binding protein), which normally binds to the H-chain in the Endoplasmic Reticulum (ER) during folding and assembly, until it is displaced by the L-chain. Because of the V_{HHS} 's increased hydrophilicity, secretion from the ER is improved.

Within certain embodiments, functional V_{HHS} may be obtained from proteolysed HCAB of an immunized camelid, by direct cloning of V_{HH} genes from B-cells of an immunized camelid resulting in recombinant V_{HHS} , or from naïve or synthetic libraries. V_{HHS} with desired antigen specificity may also be obtained through phage display methodology. Using V_{HHS} in phage display is much simpler and more efficient as compared with Fabs or scFvs, since only one domain needs to be cloned and expressed to obtain a functional antigen-binding fragment. Muyldermans, *Biotechnol.* 74:277-302 (2001); Ghahroudi *et al.*, *FEBS Lett.* 414:521-526 (1997); and van der Linden *et al.*, *J. Biotechnol.* 80:261-270 (2000).

Alternatively, ribosome display methodology may be suitably employed for the identification and isolation of scFv and/or V_{HH} molecules having the desired binding activity and affinity. Irving *et al.*, *J. Immunol. Methods* 248:31-45 (2001). Ribosome display and selection has the potential to generate and display large libraries representative of the theoretical optima for naïve repertoires (10^{14}).

Other embodiments provide V_{HH}-like molecules generated, through the process of camelisation, by modifying non-*Camelidae* V_{HS}, such as human V_{HS}, to improve their solubility and prevent non-specific binding, by replacing residues on the V_L side of V_{HS} with V_{HH}-like residues, thereby mimicking the more soluble V_{HH} fragments. Camelised V_H fragments, particularly those based on the human framework, are expected to exhibit a greatly reduced immune response when administered *in vivo* to a patient and, accordingly, are expected to have significant advantages for therapeutic purposes. Davies *et al.*, *FEBS Lett.* 339:285-290 (1994); Davies *et al.*, *Protein Eng.* 9:531-537 (1996); Tanha *et al.*, *J. Biol. Chem.* 276:24774-24780 (2001); and Riechmann *et al.*, *Immunol. Methods* 231:25-38 (1999).

A wide variety of expression systems are available in the art for the production of anti-FGFR5 antibody fragments including Fab fragments, scFv, and V_{HHS}. For example, suitable to the large-scale production of antibody fragments and antibody fusion proteins are expression systems of both prokaryotic and eukaryotic origin. Particularly advantageous are expression systems that permit the secretion of large amounts of antibody fragments into the culture medium.

Eukaryotic expression systems for large-scale production of antibody fragments and antibody fusion proteins have been described that are based on mammalian cells, insect cells, plants, transgenic animals, and lower eukaryotes. For example, the cost-effective, large-scale production of antibody fragments can be achieved in yeast fermentation systems. Large-scale fermentation of these organisms is well known in the art and is currently used for bulk production of several recombinant proteins. Yeasts and filamentous fungi are accessible for genetic modifications and the protein of interest may be secreted into the culture medium. In addition, some of the products comply with the GRAS (Generally Regarded as Safe) status – they do not harbor pyrogens, toxins, or viral inclusions.

The methylotrophic and other yeasts like *Candida boidinii*, *Hansenula polymorpha*, *Pichia methanolica*, and *Pichia pastoris* are well known systems for the production of heterologous proteins. High levels of proteins in milligram to gram quantities can be obtained and scaling up to fermentation for industrial applications is possible.

The *P. pastoris* system is used in several industrial-scale production processes. For example, the use of *Pichia* for the expression of scFv fragments as well as recombinant antibodies and fragments thereof have been described. Ridder *et al.*, *Biotechnology* 13:255-260 (1995); Anadrade *et al.*, *J. Biochem (Tokyo)* 128:891-895 (2000); Pennell *et al.*, *Res. Immunol.* 149:599-603 (1998). In shake-flask cultures, levels of 250 mg/L to over 1 g/L of scFv or V_{HH} can be achieved. Eldin *et al.*, *J. Immunol. Methods* 201:67-75 (1997); Freyre *et al.*, *J. Biotechnol.* 76:157-163 (2000).

Similar expression systems for scFv have been described for *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Yarrowia lipolytica*, and *Kluyveromyces lactis*. Horwitz *et al.*, *Proc. Natl. Acad. Sci. USA* 85:8678-8682 (1988); Davis *et al.*, *Biotechnology* 9:165-169 (1991); and Swennen *et al.*, *Microbiology* 148:41-50 (2002). Filamentous fungi, such as *Trichoderma* and *Aspergillus*, have the capacity to secrete large amounts of proteins. This property may be exploited for the expression of scFv and V_{HHS}. Radzio *et al.*, *Process-biochem.* 32:529-539 (1997); Punt *et al.*, *Trends Biotechnol.* 20:200-206 (2002); Verdoes *et al.*, *Appl. Microbiol. Biotechnol.* 43:195-205 (1995); Gouka *et al.*, *Appl. Microbiol. Biotechnol.* 47:1-11 (1997); Ward *et al.*, *Biotechnology* 8:435-440 (1990); Archer *et al.*, *Antonie Van Leeuwenhoek* 65:245-250 (1994); Durand *et al.*, *Enzyme Microb. Technol.* 6:341-346 (1988); Keranen *et al.*, *Curr. Opin. Biotechnol.* 6:534-537 (1995); Nevalainen *et al.*, *J. Biotechnol.* 37:193-200 (1994); Nyyssonen *et al.*, *Biotechnology* 11:591-595 (1993); and Nyyssonen *et al.*, PCT WO 92/01797 (1992).

The following examples are offered by way of illustration, not limitation.

Example 1

ISOLATION OF cDNA SEQUENCES FROM

MURINE LYMPH NODE STROMAL CELL EXPRESSION LIBRARIES

The cDNA sequences of the present invention were obtained by high-throughput sequencing of cDNA expression libraries constructed from murine *fsn* ^{-/-} lymph node stromal cells as described below.

cDNA Libraries from Lymph Node Stromal Cells (MLSA and MLSE)

Lymph nodes were removed from flaky skin *fsn* ^{-/-} mice, the cells dissociated and the resulting single cell suspension placed in culture. After four passages, the cells were harvested. Total RNA, isolated using TRIzol Reagent (BRL Life Technologies, Gaithersburg, MD), was used to obtain mRNA using a Poly(A) Quik mRNA isolation kit (Stratagene, La Jolla, CA), according to the manufacturer's specifications. A cDNA expression library (referred to as the MLSA library) was then prepared from the mRNA by Reverse Transcriptase synthesis using a Lambda ZAP Express cDNA library synthesis kit (Stratagene, La Jolla, CA). A second cDNA expression library, referred to as the MLSE library, was prepared exactly as above except that the cDNA was inserted into the mammalian expression vector pcDNA3 (Invitrogen, Carlsbad CA).

The nucleotide sequence of a cDNA clone isolated from the MLSA library is given in SEQ ID NO: 1, with the corresponding amino acid sequence being provided in SEQ ID NO: 5.

Example 2

CHARACTERIZATION OF ISOLATED cDNA SEQUENCES

The isolated cDNA sequences were compared to sequences in the EMBL DNA database using the computer algorithm BLASTN, and the corresponding polypeptide sequences (DNA translated to protein in each of 6 reading frames) were compared to sequences in the SwissProt database using the computer algorithm BLASTP. Specifically, comparisons of DNA sequences provided in SEQ ID NO: 1-4 to sequences in the EMBL (Release 60, September 1999) DNA database, and amino acid sequences

provided in SEQ ID NO: 5-8 to sequences in the SwissProt and TrEMBL (up to October 20, 1999) databases were made as of December 31, 1999. The cDNA sequences of SEQ ID NO: 1-4, and their corresponding polypeptide sequences (SEQ ID NO: 5-8, respectively) were determined to have less than 75% identity (determined as described above) to sequences in the EMBL and SwissProt databases using the computer algorithms BLASTN and BLASTP, respectively..

Using automated search programs to screen against sequences coding for known molecules reported to be of therapeutic and/or diagnostic use, the isolated polynucleotides of SEQ ID NO: 1-4 were determined to encode polypeptide sequences that are members of the fibroblast growth factor (FGF) receptor family (SEQ ID NO: 5-8). A family member is herein defined to have at least 20% identical amino acid residues in the translated polypeptide to a known protein or member of a protein family.

Fibroblast growth factor receptors belong to a family of four single membrane-spanning tyrosine kinases (FGFR1 to 4). These receptors serve as high-affinity receptors for 23 growth factors (FGF1 to 23). FGF receptors have important roles in multiple biological processes, including mesoderm induction and patterning, cell growth and migration, organ formation and bone growth (Xu, *Cell Tissue Res.* 296:33-43, 1999). Further analysis of the sequence revealed the presence of a putative transmembrane domain and intracellular domain, similar to other FGF receptors.

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Example 3

ISOLATION OF FULL LENGTH CDNA SEQUENCE OF A MURINE FIBROBLAST

GROWTH FACTOR RECEPTOR HOMOLOG

The full-length cDNA sequence of a murine fibroblast growth factor receptor homolog was isolated as follows.

The MLSA cell cDNA library (described in Example 1) was screened with an [α ³²P]-dCTP labeled cDNA probe corresponding to nucleotides 1 to 451 of the coding region within SEQ ID NO: 1. Plaque lifts, hybridization and screening were performed using standard molecular biology techniques. The determined polynucleotide sequence of the full-length murine FGFR gene (referred to as muFGFR5 β) is provided in SEQ ID NO: 2, with the corresponding polypeptide sequence being provided in SEQ ID NO: 6.

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Analysis of the polynucleotide sequence of SEQ ID NO: 2 revealed the presence of a putative transmembrane domain encoded by nucleotides 1311 to 1370. The polypeptide sequence (SEQ ID NO: 6; Figure 1) has regions similar to the extracellular domain of the fibroblast growth factor receptor family. The amino acid sequence of the extracellular domain of muFGFR5 β is provided in SEQ ID NO: 13, while the amino acid sequence of the intracellular domain is provided in SEQ ID NO: 14.

A splice variant of SEQ ID NO: 2 was also isolated from the MLSA cDNA library as described in Example 1. The determined polynucleotide sequence of the splice variant (referred to as FGFR5 γ) is provided in SEQ ID NO: 3 and the corresponding polypeptide sequence is provided in SEQ ID NO: 7. The splice regions are in an equivalent position to splice sites for previously described FGF receptors (Ornitz, *J. Biol. Chem.* 296:15292-15297 (1996); Wilkie, *Current Biology* 5:500-507 (1995); Miki, *Proc. Natl. Acad. Sci. USA* 89:246-250 (1992), thus establishing that this molecule (referred to herein as FGFR5) is a FGF receptor homolog. The main difference between the two FGFR5 splice variants is that muFGFR5 β contains three extracellular Ig-domains, while FGFR5 γ contains only two such domains.

To examine the structural similarities between FGFR5 γ and FGFR5 β and the other members of the FGF receptor family, 3D Swiss modeller (Petisch, *Bio/Technology* 13:658-660 (1995); Peitsch, *Biochem Soc Trans.* 24:274-279 (1996); and Guex and Peitsch, *Electrophoresis* 18:2714-2723 (1997)) was employed to produce a predicted crystal structure of the extracellular domain of FGFR5 γ . These studies showed that the crystal structure of FGFR5 deviates from that of the known FGFR1 structure between residues 188 and 219 of SEQ ID NO: 7 (SEQ ID NO: 15). These residues correlate with an area of low homology between FGFR5 and other members of the FGF receptor family that may have a critical role in defining ligand specificity.

The critical residues for ligand binding have previously been identified in co-crystallization studies of FGFR1 binding FGF-2 (Plotnikov et al., *Cell* 98:641-650 (1999)). Alignment of FGFR5 γ with FGFR1 showed that many of these residues are conserved or are a conservative substitution. Conserved ligand binding residues between the two receptors are found at residues 66, 68, 146, 178, 181, 183 and 216 of SEQ ID NO: 7, while conservative substitutions of potential ligand binding residues are found at

residues 64, 180 and 226 of SEQ ID NO: 7. When visualized on the predicted crystal structure of FGFR5 γ , these residues line the groove of the ligand binding domain. Thus, while the overall degree of similarity between FGFR5 and other FGF receptors (*i.e.* FGFR 1-4) is relatively low, the extracellular domains of the FGFR5 splice variants have
5 all the conserved residues important for ligand binding.

The main difference between the FGFR5 receptor and other family members is the lack of an intracellular tyrosine kinase domain. With the four previously identified FGF receptors (FGFR1-4), signal transduction is mediated by ligand binding and receptor dimerization, resulting in autophosphorylation of the tyrosine residues within the
10 intracellular RTK domain and phosphorylation of a number of intracellular substrates, initiating several signal transduction cascades. The FGFR5 β and FGFR5 γ splice variants described herein both contain tyrosine residues in the intracellular domain demonstrating similarity to a SHP binding motif (residues 458-463 of SEQ ID NO: 6 and 367-377 of
15 SEQ ID NO: 7). SHPs are protein tyrosine phosphatases that participate in cellular signalling and that have previously been identified in the cytoplasmic domains of many receptors eliciting a broad range of activities. The presence of such motifs in the cytoplasmic domain of FGFR5 is thus indicative of signalling, and modification of these motifs may be employed to modulate signal transduction initiated by binding of a ligand to FGFR5. These motifs are conserved between the mouse FGFR5s and the human
20 homologs described below (Example 4). Removal or modification of these signaling motifs and/or the cytoplasmic domain of FGFR5 may be employed to engineer a soluble FGFR5-like molecule that binds to the FGFR5 ligand without stimulating signaling. Such a molecule may be usefully employed to modulate the binding, and therefore activity, of FGFR5.

25

Example 4

ISOLATION OF A HUMAN FGF RECEPTOR HOMOLOG

The cDNA encoding the partial murine FGF receptor (SEQ ID NO: 1) was used to search the EMBL database (Release 58, March 1999) to identify human EST
30 homologs. The identified EST (Accession Number AI245701) was obtained from Research Genetics, Inc (Huntsville AL) as I.M.A.G.E. Consortium clone ID 1870593.

Sequence determination of the complete insert of clone 1870593 resulted in the identification of 520 additional nucleotides. The insert of this clone did not represent the full-length gene. The determined nucleotide sequence of the complete insert of clone 1870593, which represents the extracellular domain of the human FGF receptor homolog, is given in SEQ ID NO: 4 and the polypeptide sequence of the extracellular domain in SEQ ID NO: 8. Several conserved domains were identified in SEQ ID NO: 8 that are involved in the dimerization, ligand binding and activity of the receptor. These are shown in Figure 10.

Both murine and human FGFR5 are structurally similar to FGFR1-4, the other members of the FGFR family. In the extracellular domain, three immunoglobulin-like motifs are present that are flanked by conserved cysteine residues. The Ig-1 loop is the least conserved of the three Ig loops and is not required for ligand binding, but regulates binding affinity (Shi *et al.*, *Mol. Cell. Biol.* 13:3907-3918 (1993)). The Ig-3 loop is involved in ligand selectivity (Ornitz *et al.*, *Science* 268:432-436 (1996)).

An acidic box is characteristic in FGFR1-4 and is involved in binding divalent cations, including copper and calcium. Acidic boxes are important for interaction with cell adhesion molecules, extracellular matrix and heparin (Patstone and Maher, *J. Biol. Chem.* 271:3343-3346 (1996)). The acidic box in FGFR5 is smaller than in the other four receptors or absent.

The cell adhesion molecule (CAM) homology and heparin-binding domain is also characteristic of the extracellular domain (Szebenyi and Fallon, *Int. Rev. Cytol.* 185:45-106 (1999)). The CAM homology region is a binding site for L1, N-CAM and N-cadherin (Doherty *et al.*, *Perspect Dev Neurobiol.* 4(2-3):157-68 (1996)).

The FGFR5 heparin-binding domain is typical of other FGFR heparin-binding domains and consists of a cluster of basic and hydrophobic residues flanked by Lys residues (Kan *et al.*, *Science* 259:1918-1921 (1993)). Heparin or heparan sulfate proteoglycans are essential co-factors for the interaction of FGFs with FGFRs and it has been shown that heparin is a growth-factor independent ligand for FGFR4 (Gao and Goldfarb, *EMBO J.* 14:2183-2190 (1995)).

Example 5CHARACTERIZATION OF THE MURINE FGF RECEPTOR HOMOLOG

Soluble forms of the murine FGF receptor homolog muFGFR5 β and splice variant FGFR5 γ (SEQ ID NOs: 2 and 3, respectively) were expressed in mammalian cells and the purified proteins used to determine the ligand binding specificity of the receptor molecules as follows.

The extracellular domains of muFGFR5 β and FGFR5 γ were amplified by PCR using primers MS158 and MS159 (SEQ ID NOs: 10 and 11, respectively) and cloned into the expression vector pcDNA3 containing the Fc fragment from human IgG1. These soluble recombinant proteins, referred to as FGFR5 β Fc and FGFR5 γ Fc, were expressed in HEK293 cells (ATCC No. CRL-1573, American Type Culture Collection, Manassas, VA) and purified using an Affiprep protein A column (Biorad, Hercules CA).

FGF-2 (basic fibroblast growth factor) has previously been demonstrated to bind all FGF receptors but with a range of affinities. Binding of muFGFR5 β to FGF-2 was demonstrated by co-incubating the purified protein and FGF-2 in the presence of protein G Sepharose (Amersham Pharmacia, Uppsala, Sweden) and resolving complexes formed on denaturing polyacrylamide gels. FGF-2 (2 μ g) was incubated with 5 μ g FGFR5 β Fc, FGF Receptor 2 (FGFR2Fc) or unrelated protein (MLSA8790Fc) in 5 μ l protein G Fast Flow beads (Pharmacia, Uppsala, Sweden), PBS and 0.1% Triton X-100 for 60 min at 4°C. The beads were washed three times in 0.1% Triton X-100/PBS and resuspended in 20 μ l loading buffer (0.1 M DTT, 10% sucrose, 60 mM Tris.HCl pH 6.8, 5% SDS and 0.01% bromophenol blue). The samples were analysed on a 12% polyacrylamide gel. FGF-2, FGFR2Fc, FGFR5 β Fc and MLSA8790Fc (1 μ g of each) were loaded on the gel for comparison. After staining of the gel with Coomassie blue, a doublet of bands were visible in the lane containing FGFR5 β Fc, indicating that a complex formed between the FGF-2 and the murine FGF receptor homolog FGFR5 β Fc, and that FGF-2 is a ligand for the novel FGF receptor homolog. A doublet was also observed in the lane containing the FGFR2Fc, which was the positive control. No doublet was observed in the negative control lane containing the MLSA8790Fc protein.

The binding specificity of the murine FGF receptor homolog FGFR5 β Fc was further examined by repeating the experiment described above, replacing the FGF-2 with another known growth factor, epidermal growth factor (EGF). In this experiment, EGF did not bind to FGFR2Fc, FGFR5 β Fc or MLSA8790Fc, indicating that binding of FGF-2 to the murine FGF receptor homolog FGFR5 β Fc was specific. Similarly, in subsequent experiments employing FGF-7, no binding of FGFR2Fc, FGFR5 β Fc or MLSA8790Fc was observed.

To determine the difference in binding affinity between FGFR5 and FGFR2, the ability of FGFR5 β Fc and FGFR5 γ Fc to inhibit FGF signalling in FGF-responsive NIH-3T3 SRE reporter cells was examined. Fibroblast growth factors typically signal via phosphorylation of the receptor tyrosine kinase domain stimulating the MAP kinase pathway. This eventually leads to activation of genes under the control of the serum response element (SRE). Reporter constructs containing concatamerized SRE sequences upstream of a luciferase reporter gene were stably transfected into NIH-3T3 cells. Reporter activity was measured by measuring luciferase levels. As shown in Figure 2A, a dose dependent response of NIH-3T3 SRE cells to FGF-2 was seen in the presence of heparin. Using a standard dose of FGF-2 in the presence of heparin, an increasing concentration of FGFR2Fc, FGFR5 β Fc or FGFR5 γ Fc was titrated onto the NIH-3T3 SRE cells and luciferase activity was measured. Increasing concentrations of FGFR2Fc, the positive control, reduced the luciferase signal in FGF-2 stimulated cells (Figure 2B). However, titrating FGFR5 β Fc and FGFR5 γ Fc did not inhibit FGF-mediated luciferase signal from the NIH-3T3 SRE cells. These results show that FGF-2 has lower affinity for either FGFR5 β or FGFR5 γ than for FGFR2, and indicate that the ligand specificity of FGFR5 is different to those of the other members of the FGF receptor family.

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Example 6

SEQUENCE DETERMINATION OF A POLYNUCLEOTIDE FRAGMENT CONTAINING GENOMIC MURINE FGFR5 β

As noted above, the two splice variants muFGFR5 β and muFGFR5 γ do not contain the classical receptor tyrosine kinase domain present in other known FGF

30

receptors. In order to investigate the existence of a splice variant of FGFR5 containing a classical receptor tyrosine kinase (RTK) domain, the genomic DNA of FGFR5 was cloned and sequenced as follows.

Mouse genomic DNA was isolated from L929 cells using standard techniques. A genomic polynucleotide fragment containing murine FGFR5 β was PCR amplified using primers MS157 and MS166 (SEQ ID NOs: 11 and 12, respectively). The 1.4 kb polynucleotide fragment was cloned into a T-tailed pBluescript SK²⁺ vector. The sequence of the insert of this plasmid was determined using standard primer walking sequencing techniques. The sequence of the genomic fragment containing murine FGFR5 β is given in SEQ ID NO: 9. This sequence extends from the 3' untranslated region to the sequence encoding the 5' end of the mature FGFR5 receptor minus the signal sequence. No alternative exons expressing an RTK domain were identified.

Example 7

STIMULATION OF CELL GROWTH BY MURINE FGFR5 β AND FGFR5 γ

RAW264.10 cells are derived from a murine macrophage cell line generated from BALB/c mice, and are macrophage and osteoblast precursors. Stimulation of RAW264.10 cells (Hamilton *et al.*, *J. Exp. Med.* 148:811-816 (1978)) and peripheral blood mononuclear cells (PBMC) in the presence of the murine FGFR5 β and FGFR5 γ (also referred to herein as FGFR β and FGFR γ , respectively) was demonstrated as follows.

The murine FGF receptor homolog, muFGFR5 β , and splice variant FGFR5 γ (SEQ ID NOs: 2 and 3, respectively) were expressed in mammalian cells and purified as murine FGFR5 β -Fc and FGFR5 γ -Fc fusion proteins as described above. The FGFR5 β - and FGFR5 γ -Fc fusion proteins were titrated from 10 nM in 0.05 ml media (DMEM supplemented with 5% FBS, 2mM L-glutamine (Sigma, St Louis MO), 1 mM sodium pyruvate (Life Technologies, Gibco BRL, Gaithersburg MD), 0.77 mM L-asparagine (Sigma), 0.2 mM arginine (Sigma), 160 mM penicillin G (Sigma), 70 mM dihydrostreptomycin sulfate (Boehringer Mannheim, Roche Molecular Biochemicals, Basel, Switzerland) in a 96-well flat-bottomed microtitre plate. Purified human FGFR2-Fc fusion protein was used as control and titrated from 10 nM.

RAW264.10 cells were added to each well in 0.05 ml media at a concentration of 2×10^4 cells/ml. The plate was incubated at 37°C in a humidified atmosphere containing 10% CO₂ for 4 days. Cell growth was determined by MTS dye conversion and quantified using an ELISA reader. As shown in Figure 3, both murine FGFR5 β -Fc and FGFR5 γ -Fc fusion proteins stimulated the growth of RAW264.10 cells at concentrations of 100 pM and greater of Fc fusion protein.

These results demonstrated that FGFR5 β and FGFR5 γ are immunostimulatory molecules that directly activate a macrophage cell line. The macrophage cell line used in these assays (RAW264.10) has previously been shown to differentiate into osteoblasts when stimulated with a variety of known bone morphogenic agents. The effects of FGFR5 β and FGFR5 γ on these cells suggest that these molecules may also stimulate the differentiation and activation of osteoblasts. Weidemann and Trueb (*Genomics* 69:275-279 (2000)), have shown that FGFR5 is expressed in cartilaginous tissues. When viewed in the context of the data provided above, this suggests that FGFR5 may play a role in bone formation and may therefore have applications in fracture repair and bone diseases, such as osteoporosis and osteoporosis.

Example 8

STIMULATION OF PROLIFERATION AND ADHERENT PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) BY MURINE FGFR5 β AND FGFR5 γ

Stimulation of PBMC to adhere to plastic by murine FGFR5 β and FGFR5 γ Fc fusion proteins was demonstrated as follows.

Purified FGFR5 β -Fc and FGFR5 γ -Fc fusion proteins were titrated from 20 nM into 0.1 ml media per well of 96 well microtiter plates. Purified human FGFR2-Fc fusion protein and human IgG Fc were used as controls. PBMC were harvested from blood by density gradient centrifugation and resuspended in media to a concentration of 2×10^6 cells/ml. Phytohaemagglutinin (PHA), Pokeweed mitogen (PWM), anti-CD3 antibody or media was added to the PBMC and 0.1 ml of cells dispensed to each well. The plates were incubated for 3 days at 37°C in a humidified atmosphere containing 5% CO₂ in air. Cell proliferation was quantified by pulsing the plates with tritiated (³H)-thymidine for the final 16 hours of culture. The cells were then harvested and ³H-thymidine

incorporation quantified by standard liquid scintillation counting. Figures 4-6 show that murine FGFR5 β and FGFR5 γ fusion proteins enhanced proliferation of PBMCs activated with either PHA or anti-CD3 but did not induce the proliferation of PBMC on their own. Proliferation was not stimulated with human FGFR2-Fc fusion protein or human IgG Fc.

5 MuFGFR5 β and muFGFR5 γ (SEQ ID NO: 2 and 3, respectively) were expressed in mammalian cells and purified as Fc fusion proteins as described above. The muFGFR5 β -Fc and muFGFR5 γ -Fc fusion proteins were titrated from 10 nM into 0.1 ml media per well of 96 well microtitre plates. Peripheral blood mononuclear cells (PBMC) were harvested from blood by density gradient centrifugation and resuspended in media
10 to a concentration of 2×10^6 cells/ml. PHA or media (RPMI 1640 supplemented with 5% FBS, 2 mM L-glutamine (Sigma), 160 mM penicillin G (Sigma), and 70mM dihydrostreptomycin sulfate (Boehringer Mannheim) was added to the PBMC and 0.1 ml of cells dispensed to each well. The plates were incubated for 3 days at 37°C in a humidified atmosphere containing 5% CO₂ in air. The non-adherent cells were removed
15 with three media washes. Media (0.05 ml) containing MTS/PES solution (CellTiter96 Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI) was dispensed to each well and the plate incubated for 4 hrs before the degree of dye conversion was quantified using a 96-well ELISA plate reader. Figures 7 and 8 show that muFGFR5 β and muFGFR5 γ Fc fusion proteins stimulated, in a dose dependent manner, the adherence
20 of PBMC as well as the proliferation of the adherent PBMC. Furthermore, PHA stimulation augmented this effect. These results demonstrate that FGFR5 β and FGFR5 γ are capable of enhancing the proliferative effects of known immunostimulatory molecules on a mixed population of human haemopoietic cells, namely PBMC.

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Example 9

ACTIVATION OF NATURAL KILLER CELLS BY MURINE FGFR5 β AND FGFR5 γ

This Example discloses the activation of Natural Killer (NK) cells by muFGFR5 β -Fc and muFGFR5 γ -Fc fusion proteins.

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Peripheral blood mononuclear cells (PBMC) were harvested from blood by density gradient centrifugation and resuspended in media (RPMI 1640 supplemented with

5% FBS, 2 mM L-glutamine (Sigma), 160 mM penicillin G (Sigma), 70 mM dihydrostreptomycin sulfate (Boehringer Mannheim)) to a concentration of 2×10^6 cells/ml. Purified muFGFR5 β -Fc and muFGFR5 γ -Fc fusion proteins were added to the cells at a concentration of 10 nM and the cells were cultured in 6 well plates (3 ml/well) for 3 days at 37°C in a humidified atmosphere containing 5% CO₂ in air. Purified human FGFR2-Fc fusion protein was used as control. The non-adherent cells were removed with three media washes. The adherent cells were collected by light trypsinization and scraping. The cells were washed into staining buffer and their phenotype determined by standard flow cytometric techniques using the NK cell marker CD56 and a control isotype antibody.

As shown in Figure 9, muFGFR5 β -Fc and muFGFR5 γ -Fc fusion proteins stimulated the adherence and/or growth of adherent cells from human PBMC, with approximately 50% of these cells being NK cells. The filled histograms represent the adherent PBMC stained with the NK cell marker CD56 and the open histograms represent the same cells stained with the isotype-matched control antibody. FGFR2 did not stimulate the adherence of PBMC and therefore there were no cells to analyze from these cultures. These results demonstrate that FGFR5 β and FGFR5 γ are immunostimulatory molecules that directly activate NK cells. These results, plus those provided in Example 8, above, demonstrated that FGFR5 can enhance immune responses, and may therefore be usefully employed to enhance vaccine responses and anti-cancer therapies.

Example 10

STIMULATION OF GENE EXPRESSION IN HUMAN MONOCYTES BY

MURINE FGFR5 β -Fc FUSION PROTEIN

This Example discloses genes that were overexpressed in human monocytes stimulated with the murine FGFR5 β -Fc fusion protein.

Monocytes were purified from human peripheral blood mononuclear cells (PBMC) by adherence for 2 hours at 37°C. Cells were stimulated with 100 nM of soluble FGFR5 β human IgG Fc fusion protein or soluble FGFR2 human IgG Fc fusion protein. After 0 and 12 hours the adherent monocytes were collected and total RNA extracted

from the cells using Trizol reagent (Invitrogen Corp., Carlsbad CA) following the manufacturer's instructions. The RNA was amplified and aminoallyl UTP incorporated using the Ambion MessageAmp aRNA kit (Ambion Inc, Austin TX) following the manufacturer's instructions.

5 The extracted amplified RNA from the FGFR5 β and FGFR2-treated cells was labelled with either Cy3 or Cy5 dye (Amersham Pharmacia Biotech, Buckinghamshire UK), respectively, by indirect aminoallyl dUTP labeling and hybridized to 2 Clontech Atlas Glass 3.8 gene microarrays (BD Biosciences Clontech, Palo Alto, CA). The slides were washed, scanned and analyzed using Axon GenePix scanner and software (Axon
10 Instruments Inc., Union City, CA). Where indicated, quantitative PCR was used to validate the microarray data and quantify the mRNA for genes not present on the array. Primers and probe sets were purchased from Perkin Elmer/Applied Biosystems (Foster City, CA) and MWB Biotech (Ebersberg, Germany) and all PCR reactions were run on a Perkin Elmer/Applied Biosystems 7700 following the manufacturer's instructions.

15 Treatment of monocytes with FGFR5 β -Fc up-regulated expression of the 26 genes listed in Table 1 below. The up-regulation of three of the genes was confirmed by quantitative PCR. In addition, the expression of eight human cytokines was analyzed by quantitative PCR and the results of this analysis are shown in Table 1.

20 FGFR5-Fc stimulated a dramatic up-regulation in the levels of osteopontin (OPN) and TGF β but had only modest effects on the other cytokines. This profile of gene expression was very unlike that described for other stimulators of monocytes such as LPS, *Mycobacterium tuberculosis*, GM-CSF and M-CSF, which stimulate modest OPN expression but pronounced expression of pro-inflammatory cytokines such as IL-1 β , IL-6, IL-8 IL-10, IL-12 and TNF α (Rosenberger *et al.*, *J. Immunol.* 164:5894-904 (2000);
25 Suzuki *et al.*, *Blood* 96:2584-2591 (2000); Hashimoto *et al.*, *Blood* 94:837-844 (1999); Hashimoto *et al.*, *Blood* 94:845-852 (1999); Boldrick *et al.*, *Proc. Natl. Acad. Sci. USA* 99:972-977 (2002); Ragno *et al.*, *Immunol.* 104:99-108 (2001)).

Table 1: Genes up-regulated in monocytes following treatment with FGFR5

		Microarray	Quantitative PCR
	GENBANK	Fold up-regulation	Fold up-regulation
<i>Secreted Molecules</i>			

Osteopontin	NM_000582	4.95	48.4
Interferon, alpha 8	NM_002170	2.27	ND
EXODUS	NM_004591	2.27	6.3
IL-1 β	XO2532	Not Determined (ND)	3.4
IL8	NM_000584	ND	5.5
IL-10	NM_000572	ND	Undetectable
IL-12 p35	NM_000882	ND	Undetectable
IL-12p40	NM_002187	ND	Undetectable
IL-20	NM_018724	ND	Undetectable
TGF β	NM_000660	ND	27.3
TNF α	XO1394	ND	4.0
Channels and Receptors			
MICA	NM_000247	2.08	4.7
TIE1	NM_005424	3.30	ND
Calcium channel, voltage-dependent, beta 4 subunit	NM_000726	2.44	ND
LDL receptor-related protein 8	NM_004631	2.20	ND
Cytoskeletal Molecules			
Myosin VI	NM_004999	1.89	ND
Myosin, heavy polypeptide 1	NM_005963	2.12	ND
Troponin C, slow	NM_003280	1.88	ND
Kinectin 1 kinesin receptor	NM_004986	1.73	ND
Signalling Molecules			
Protein kinase C, iota	NM_002740	2.26	ND
Protein tyrosine phosphatase, non-receptor type 9 MEG-2	NM_002833	1.85	ND
Importin alpha 6	NM_002269	2.17	ND
Protein kinase, X-linked	NM_005044	1.92	ND
Suppression of tumorigenicity 5	NM_005418	3.16	ND
RAR-related orphan receptor B	NM_006914	2.08	ND
Zinc finger protein 124 HZF-16	NM_003431	2.94	ND
Metabolism			
Ubiquitin-conjugating enzyme	NM_003341	2.41	ND
Transplantation antigen P35B	NM_003313	2.48	ND
UDP glycosyltransferase 2	NM_001075	2.35	ND
Alcohol dehydrogenase 2	NM_000668	2.41	ND
Solute carrier family 18 vesicular monoamine, member 1	NM_003053	2.07	ND
Seryl-tRNA synthetase	NM_006513	1.88	ND
Other			
H1 histone family, member 1	NM_005325	1.99	ND
Chr. 8 open reading frame 1	NM_004337	2.08	ND

In addition to demonstrable upregulation of OPN mRNA, PBMC and adherent PBMC (predominantly monocytes) were stimulated with FGFR2, FGFR5, LPS or media alone for 24 hours and the supernatants collected for cytokine analysis. LPS induced the production of expected pro-inflammatory cytokines such as IL-1, IL-6, and TNF α whereas FGFR5 did not. In contrast, FGFR5 stimulated both PBMC and adherent PBMC to produce 90 and 130 ng/ml of osteopontin, respectively. LPS stimulated 20 and 50 ng/ml of osteopontin and FGFR2 and the media control cultures contained less than 20 ng/ml of OPN. See, Figure 11A-C. These results are consistent with the microarray and real time PCR results presented in Table 1, above, and demonstrate that FGFR5 selectively stimulated osteopontin production by PBMC.

In total, the results presented herein demonstrate that FGFR5 is a potent, and almost specific, stimulator of osteopontin expression. Osteopontin (OPN) is a multifunction protein secreted by activated macrophages that shares most of the functions described herein for FGFR5. More specifically, OPN is a potent immunostimulatory molecule (O'Regan *et al.*, *Immunol. Today* 21:475-478 (2000)) that stimulates macrophage adherence, activation, cytokine secretion and growth. It has been shown that OPN is a regulator of T-cell responses in that it augments CD3-induced proliferation, IFN γ production, and CD40 ligand expression. OPN also enhances Th1 and inhibits Th2 cytokine expression. It directly induces macrophages to produce IL-12 and inhibits IL-10 expression by LPS stimulated macrophages (Ashkar *et al.*, *Science* 287:860-864 (2000)). OPN has also been shown to induce B cell proliferation and auto-reactive antibody production and it appears that OPN may preferentially activate a CD5+ subset of B-cells and induce the production of auto-antibodies.

Osteopontin has been linked with a number of pathophysiological states including a variety of tumors; autoimmune diseases such as multiple sclerosis (MS), systemic lupus erythematosus (SLE), diabetes and rheumatoid arthritis; granulomatous inflammation such as sarcoidosis and tuberculosis; and pathological calcifications such as kidney stones and atherosclerosis (Giachelli and Steitz, *Matrix Biol.* 19:615-622 (2000)). Elevated levels of OPN are found in the sera of SLE patients and the autoimmune-prone MRL mice. Recently two groups described a central role for OPN in multiple sclerosis (Chabas *et al.*, *Science* 294:1731-1735 (2001) and Jansson, *J. Immunol.* 168:2096-2099 (2002)).

OPN is prevalent in the plaques of MS patients and, due to its immunostimulatory properties, it has been proposed that OPN plays a role in the progression of MS. This effect was demonstrated in experimental allergic encephalopathy (EAE), the murine model for MS. Mice that lacked the OPN gene were resistant to progressive EAE and
 5 had frequent remissions when compared to wild-type mice expressing OPN.

SLE is an autoimmune disorder that affects 24 out of 100,000 individuals in the USA. Afflicted individuals usually develop nephritis, arthritis and dermatitis. Auto-antibody production, complement activation, immune complex deposition, Fc receptor ligation and leukocyte infiltration of the target organs are among the immunopathogenic
 10 events. The chromosomal location of FGFR5 is 4p16. Genetic screens on large numbers of SLE patients show that a mutation at this location is associated with disease. FGFR5 sequence analysis may thus be used to identify individuals at risk for SLE by determining whether a mutation exists.

OPN has also been shown to function in bone remodelling by inhibiting
 15 calcification. Inhibition of OPN expression, by reducing the level or binding of FGFR5, may thus be useful in the treatment of osteoporosis or bone fractures. Conversely, FGFR5, or an agent that augments FGFR5 levels or activity, may be of benefit to patients who suffer from conditions that cause excessive bone formation such as osteopetrosis.

A number of recent studies have shown that OPN is overexpressed in many forms
 20 of cancer including breast cancers, hepatocellular carcinomas and colon cancers. Furger et al., Curr. Mol. Med. 1:621-632 (2001); Tuck and Chambers, J. Mammary Gland Biol. Neoplasia 6:419-429 (2001); Yeatman and Chambers Clin. Exp. Metastasis 20:85-90 (2003), Ye et al., Nat. Med. 4:416-423 (2003). OPN expression appears to be linked to the malignant phenotype of cancers via induction of cell motility and invasion and
 25 changes in expression of genes that contribute to malignant behaviour. FGFR5 may drive the expression of OPN in tumours and therefore an inhibitor of FGFR5 expression or antagonist of FGFR5 function and/or activity could act as a cancer therapeutic.

Many of the effects described for FGFR5 may be mediated by its ability to induce high levels of osteopontin expression. Osteopontin is clearly a key molecule in the
 30 progression of a number of disease processes and therefore regulators of osteopontin

expression, such as FGFR5, are targets for therapeutics for osteopontin-mediated diseases, including SLE, vasculitis, atherosclerosis, nephritis and arthritis.

Example 11

5 ANALYSIS OF FGFR5 EXPRESSION USING FGFR5 SPECIFIC ANTIBODIES

This example discloses the preparation of a rabbit anti-FGFR5 polyclonal antisera and its utility in detecting the expression of FGFR5 protein in a variety of normal and disease tissues from humans.

10 Polyclonal antibodies were generated to the extracellular domain of FGFR5 β by immunizing rabbits with murine FGFR5 β extracellular domain fused to human IgG1 Fc fragment emulsified in complete Freund's adjuvant. The FGFR5-specific immune response was boosted by three subcutaneous injections at weekly intervals with the same protein and then twice with pure murine FGFR5 β extracellular domain protein. Antisera were collected from the rabbits and the IgG purified by Protein A affinity
15 chromatography.

Antibodies raised to the human IgG Fc portion of the immunogen were removed by absorption to Sephadex beads coated with human IgG. The resultant polyclonal antibody specifically reacted with human and mouse FGFR5 but did not recognize human FGFR1, 2, 3, or 4 Fc fusion proteins (purchased from R&D Systems, Minneapolis MN)
20 in ELISA or by Western blotting.

Immunohistochemical analysis of human normal and diseased tissue arrays (SuperBioChips Laboratories, Seoul, Korea) revealed that FGFR5 was expressed in a minor population of granulocyte cells in the red pulp region of the spleen. These cells are likely to be granulocytes. FGFR5-expressing granulocytes were also found in a number
25 of tissues including the stomach, lung and small intestine. FGFR5 expression was also detected in skeletal muscle, skin and kidney. In addition, expression of FGFR5 was found in tissue biopsies from a hepatocellular carcinoma and a squamous cell carcinoma.

Sarcoidosis is thought to be an autoimmune disease that is characterized by the formation of non-caseating sterile granulomas. Granulomas are nodular lesions that form
30 due to chronic localized stimulation of macrophages that differentiate into large epithelioid cells, histiocytes, and giant cells.

Two human sarcoidosis patient biopsy samples were cut and stained for FGFR5 expression. The first biopsy sample was a lymph node that was filled with numerous small granulomas surrounded by lymphoid tissue. Many, but not all of the lymphoid cells expressed FGFR5 to varying degrees. Lymphoid cells associated with vessel structure were strongly FGFR5⁺ whereas other cells were found to express lower levels of FGFR5 protein. Cells within the granulomas also appeared to express very low levels of FGFR5 protein.

The second biopsy was taken from the liver and contained many small inflammatory foci that exhibited a different structure to the archetypal granuloma observed in the first biopsy sample. These foci appeared to be exemplary of granulomatous lesions. The liver cells in the second biopsy sample expressed FGFR5 protein. In contrast to the lymph node sample, fewer of the leukocytes expressed high levels of FGFR5 while all of the leukocytes present in a small, presumably emerging, lesion expressed very high levels of FGFR5.

The Sarcoidosis patient tissue biopsy samples were further analyzed with the Envision Plus Dextran complex based kit (DakoCytomation, Glostrup, Denmark). Granulomas in the lymph node expressed FGFR5 to varying degrees ranging from moderate to no expression. Some of the giant cells, present in the more mature granulomas, stained quite strongly for FGFR5 whereas the histiocytes of others stained only weakly. Scattered in amongst the granulomas were remnants of lymphoid follicles and granulocytes. The granulocytes stained intensely with the antibody whereas pockets of lymphoid cells expressed lower levels of FGFR5. These experiments demonstrated that FGFR5 was expressed in granulomas and granulocytes and may be expressed by some lymphocytes. The granuloma in the liver biopsy did not express FGFR5 and there were only scattered FGFR5⁺ granulocytes spread throughout the section. The strongly FGFR5⁺ cells were identified as granulocytes. The histocytes and giant cells in the granulomas also expressed FGFR5.

In total the results obtained with these two biopsy samples demonstrate the expression of FGFR5 in sarcoid lesions and suggest that FGFR5 may participate in fueling the disease process.

Example 12EFFECTS OF FGFR5 ADMINISTRATION *IN VIVO*

This Example discloses the effects of *in vivo* administration of FGFR5 β protein to mice.

5 Experiment 1 used BALB/cByJ mice and experiment 2 used C3H/HeJ mice. Both sets of mice were injected subcutaneously with 5 μ g (55 nM in 0.1 ml PBS) of murine FGFR5 β extracellular domain (ECD) - murine IgG3 Fc fusion protein in the morning and the same dose in the evening (*i.e.* each mouse received 10 μ g per day) for five days. Control mice received PBS alone. On the sixth day, the mice were sacrificed and the
10 draining lymph nodes (axillary and lateral axillary) were removed. A single cell suspension was generated from the lymph nodes of each mouse and the number of cells collected from each mouse was determine by trypan blue viability counting using a haemocytometer. The lymph node cells collected from the FGFR5-treated mice were then pooled. The lymph node cells collected from the PBS-treated mice were
15 amalgamated into a separate pool of cells. The cells from both the FGFR5 and PBS-treated mice were then stained for the cell surface antigens listed in Table 3, below, and analysed by flow cytometry.

In a third experiment, C3H/HeJ mice were injected subcutaneously with 10 μ g (110 nM in 0.1 ml PBS) of murine FGFR5 β ECD - human IgG1 Fc fusion protein in one
20 injection per day for 5 days. While the treatment regime differed from that used in Experiments 1 and 2 above, the total dose of protein administered to the mice was not altered. Control mice were administered human IgG1 Fc fragments alone. On the sixth day, the mice were sacrificed and the draining lymph nodes (axillary and lateral axillary) removed. The number of cells collected from each mouse and the presence of cell
25 surface antigens was determined as described above.

As shown in Table 2, *in vivo* administration of FGFR5 was found to stimulate lymphadenopathy, or enlargement of the lymph nodes. When compared to mice treated with Fc protein, the frequency of B cells doubled in the draining lymph nodes of FGFR5-treated mice. An analysis of the cell cycle state of the B cells by flow cytometry
30 indicated that they were not expanding but were either selectively migrating or being retained in the lymph nodes. This is consistent with the data provided above showing

that FGFR5 causes the growth of macrophages but not T or B cells in culture. The cells were, however, activated as there was an increase in the number of cells expressing the very early activation antigen, CD69.

5

Table 2: Comparison of Three *in vivo* experiments testing the effects of soluble FGFR5 in mice

Markers	Cell type recognized	<u>Experiment 1</u>		<u>Experiment 2</u>		<u>Experiment 3</u>	
		<u>Balb/c</u>		<u>C3H/HeJ</u>		<u>C3H/HeJ</u>	
		<u>Murine Fc FGFR5</u>	<u>PBS</u>	<u>Murine Fc FGFR5</u>	<u>PBS</u>	<u>Human Fc FGFR5</u>	<u>Human Fc</u>
CD3	T cell	63	81	59	82	32	67
<u>CD19</u>	<u>B cell</u>	35	21	39	16	61	26
Class II	B cell and macrophage	41	20	ND*	ND	ND	ND
CD45R	B cell	ND	ND	ND	ND	72	31
CD69	Activated cells	23	14	18	10	21	10

* ND = Not determined

10

Example 13

GENERATION OF MONOCLONAL ANTIBODIES DIRECTED AGAINST MURINE FGFR5

This Example discloses the preparation of murine monoclonal antibodies specific for an epitope on the murine FGFR5 extracellular domain.

Four mice were immunized with murine FGFR5 extracellular domain (ECD) fused to the murine IgG3 Fc (prepared as described above). Serum samples collected from the mice were tested for antibodies reactive to murine FGFR5. Two of the four mice were confirmed to produce anti-FGFR5 antibodies. A single mouse having the highest titer of FGFR5 antibodies was reimmunized with the FGFR5-Fc fusion protein. Splenocytes were isolated from this mouse and standard methods were employed to fuse the splenocytes to myeloma cells to generate hybridomas. After the fusion, the cells were dispensed into eighteen 96-well plates and cultured in media to select for hybridomas.

700 independent hybridoma lines were screened for FGFR5-reactive antibodies using the murine FGFR5 β ECD fused to human IgG Fc in an ELISA assay. Three independent, positive hybridomas were identified and further screened for FGFR5-specific antibodies using murine FGFR1-4 human IgG Fc fusion proteins (commercial). The
5 hybridomas specific for FGFR5 were subcloned and supernatants generated and tested in the following assays.

The three monoclonal antibodies were used to validate the FGFR5 expression profile revealed by the rabbit polyclonal antisera described herein. A series of assays revealed that all three antibodies recognized a similar epitope and competed for binding
10 to the recombinant FGFR5 protein. One of these three monoclonal antibodies was used in the following assays.

A series of immunohistochemistry experiments was performed on cells fixed to slides by cytocentrifugation. These experiments revealed that FGFR5 is expressed in the granulocytes of polymorphonuclear (PMN) leukocytes and monocytes, however, not all
15 PMN express FGFR5 with ~10% expressing little or no FGFR5, ~20% expressing moderate levels and the remainder expressing high levels of the protein. This staining pattern suggests that FGFR5 may be regulated during PMN activation and maturation.

The expression of FGFR5 in the granules of PMN and monocytes suggests that FGFR5 will be released upon activation of these cells. PMN and monocytes are key
20 drivers of inflammation and are found in a variety of disease settings where they become activated and release the contents of their granules. The granules contain many important mediators of inflammation therefore, the expression of FGFR5 in the granules supports the data on the immunomodulatory effects of this protein presented herein. Furthermore, expression of FGFR5 in the granules also suggests that FGFR5 is released in inflamed
25 tissues and may participate in driving disease pathology in a variety of diseases such, for example, as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and multiple sclerosis (MS).

SEQ ID NOS: 1-15 are set out in the attached Sequence Listing. The codes for polynucleotide and polypeptide sequences used in the attached Sequence Listing conform
30 to WIPO Standard ST.25 (1988), Appendix 2.

All references cited herein, including patent references and non-patent references, are hereby incorporated by reference in their entireties.

Although the present invention has been described in terms of specific embodiments, changes and modifications can be carried out without departing from the scope of the invention which is intended to be limited only by the scope of the appended claims.

CLAIMS

We claim:

1. A composition comprising a modulator of FGFR5 gene expression
5 wherein said modulator is selected from the group consisting of: (a) a small molecule inhibitor of gene expression, (b) an anti-sense oligonucleotide, and (c) a small interfering RNA molecule (siRNA or RNAi).
2. The composition of claim 1 wherein said modulator of FGFR5 gene
10 expression specifically binds to a polynucleotide selected from the group consisting of: (a) a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9; (b) a complement of a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9; (c) a reverse sequence of a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:
15 1-4 and 9; (d) a polynucleotide that encodes a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15; (e) a complement of a polynucleotide that encodes a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15; and (f) a reverse sequence of a polynucleotide that encodes a polypeptide comprising a sequence selected from the group consisting of:
20 SEQ ID NO: 5-8 and 13-15.
3. The composition of claim 1 or claim 2 wherein said modulator of FGFR5 gene
expression is effective in decreasing FGFR5 gene expression when contacted with a population of cells expressing FGFR5.
25
4. The composition of claim 3 wherein said modulator of FGFR5 gene
expression is effective in decreasing osteopontin gene expression when contacted with a population of cells expressing FGFR5.
5. The composition of claim 1 or claim 2 wherein said modulator of FGFR5
30 gene expression is an anti-sense oligonucleotide and wherein said anti-sense

oligonucleotide is selected from the group consisting of: (a) an anti-sense expression vector; (b) an anti-sense oligodeoxyribonucleotide, (c) an anti-sense phosphorothioate oligodeoxyribonucleotide, (d) an anti-sense oligoribonucleotide, and (e) an anti-sense phosphorothioate oligoribonucleotide.

5

6. A composition comprising a binding agent wherein said binding agent is a modulator of FGFR5 polypeptide function and wherein said binding agent is selected from the group consisting of: (a) a small molecule; (b) an antibody or antigen-binding fragment thereof; (c) a small chain antibody fragment (scFv); (d) a camelid heavy chain antibody (HCAb) or heavy chain variable domain thereof (V_{HH}); and (e) an FGFR5 ligand or antigen-binding fragment thereof.

10

7. The composition of claim 6 wherein said binding agent specifically binds to a polypeptide selected from the group consisting of: (a) a polypeptide encoded by a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9 or a complement thereof; and (b) a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15.

15

8. The composition of claim 6 or claim 7 wherein said binding agent is an agonist of FGFR5 polypeptide function.

20

9. The composition of claim 8 wherein said agonist of FGFR5 polypeptide function is effective in increasing osteopontin gene expression in a population of cells expressing FGFR5 polypeptide when said agonist is contacted with said population of cells:

25

10. The composition of claim 6 or claim 7 wherein said binding agent is an antagonist of FGFR5 polypeptide function.

30

11. The composition of claim 10 wherein said antagonist of FGFR5 polypeptide function is effective in decreasing osteopontin gene expression in a

population of cells expressing FGFR5 polypeptide when said antagonist is contacted with said population of cells.

12. A method for modulating osteopontin expression in a population of cells,
5 said method comprising the step of contacting said population of cells with the composition of claim 1.

13. The method of claim 12 wherein said modulator of FGFR5 gene
expression specifically binds to a polynucleotide selected from the group consisting of:
10 (a) a polynucleotide comprising a sequence selected from the group consisting of SEQ
ID NO: 1-4 and 9; (b) a complement of a polynucleotide comprising a sequence selected
from the group consisting of SEQ ID NO: 1-4 and 9; (c) a reverse sequence of a
polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:
1-4 and 9; (d) a polynucleotide that encodes a polypeptide comprising a sequence
15 selected from the group consisting of: SEQ ID NO: 5-8 and 13-15; (e) a complement of a
polynucleotide that encodes a polypeptide comprising a sequence selected from the group
consisting of: SEQ ID NO: 5-8 and 13-15; and (f) a reverse sequence of a polynucleotide
that encodes a polypeptide comprising a sequence selected from the group consisting of:
SEQ ID NO: 5-8 and 13-15.

20

14. The method of claim 12 wherein said modulator of FGFR5 gene
expression is effective in decreasing FGFR5 gene expression when contacted with a
population of cells expressing FGFR5.

25 15. The method of claim 12 wherein said modulator of FGFR5 gene
expression is effective in decreasing osteopontin gene expression when contacted with a
population of cells expressing FGFR5.

16. The method of claim 12 wherein said modulator of FGFR5 gene
30 expression is an anti-sense oligonucleotide and wherein said anti-sense oligonucleotide is
selected from the group consisting of: (a) an anti-sense expression vector; (b) an anti-

sense oligodeoxyribonucleotide, (c) an anti-sense phosphorothioate oligodeoxyribonucleotide, (d) an anti-sense oligoribonucleotide, and (e) an anti-sense phosphorothioate oligoribonucleotide.

5 17. A method for modulating osteopontin expression in a population of cells, said method comprising the step of contacting said population of cells with the composition of claim 6.

10 18. The method of claim 17 wherein said binding agent specifically binds to a polypeptide selected from the group consisting of: (a) a polypeptide encoded by a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9 or a complement thereof; and (b) a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15.

15 19. The method of claim 17 wherein said binding agent is an agonist of FGFR5 polypeptide function and wherein binding of said agonist to said population of cells results in an increase in osteopontin expression when said agonist is contacted with said population of cells.

20 20. The method of claim 17 wherein said binding agent is an antagonist of FGFR5 polypeptide function and wherein binding of said an antagonist to said population of cells results in a decrease in osteopontin expression when said antagonist is contacted with said population of cells.

25 21. A use of a modulator of FGFR5 gene expression in a medicament for the treatment of a disease associated with elevated osteopontin expression.

30 22. The use of a modulator of FGFR5 gene expression of claim 21 wherein said modulator is selected from the group consisting of: (a) a small molecule inhibitor of gene expression, (b) an anti-sense oligonucleotide, and (c) a small interfering RNA molecule (siRNA or RNAi).

23. The use of a modulator of FGFR5 gene expression of claim 21 wherein said modulator specifically binds to a polynucleotide selected from the group consisting of: (a) a polynucleotide comprising a sequence selected from the group consisting of
5 SEQ ID NO: 1-4 and 9; (b) a complement of a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9; (c) a reverse sequence of a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9; (d) a polynucleotide that encodes a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15; (e) a complement of a
10 polynucleotide that encodes a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15; and (f) a reverse sequence of a polynucleotide that encodes a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15.

15 24. The use of a modulator of FGFR5 gene expression of claim 21 wherein said disease associated with elevated osteopontin expression is selected from the group consisting of cancer, multiple sclerosis; systemic lupus erythematosus; diabetes; rheumatoid arthritis; sarcoidosis; tuberculosis; kidney stones; atherosclerosis; vasculitis; nephritis; arthritis; and osteoporosis.

20

25 25. A use of a binding agent in a medicament for the treatment of a disease associated with elevated osteopontin expression wherein said binding agent is an antagonist of FGFR5 polypeptide function and wherein said binding agent is selected from the group consisting of: (a) a small molecule; (b) an antibody or antigen-binding fragment thereof; (c) a small chain antibody fragment (scFv); and (d) a camelid heavy chain antibody (HCAb) or heavy chain variable domain (V_{HH}) thereof.

26. The use of a binding agent of claim 25 wherein said binding agent specifically binds to a polypeptide selected from the group consisting of: (a) a
30 polypeptide encoded by a polynucleotide comprising a sequence selected from the group

consisting of SEQ ID NO: 1-4 and 9 or a complement thereof; and (b) a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15.

27. The use of a binding agent of claim 25 wherein said disease associated
5 with elevated osteopontin expression is selected from the group consisting of cancer; multiple sclerosis; systemic lupus erythematosus; diabetes; rheumatoid arthritis; sarcoidosis; tuberculosis; kidney stones; atherosclerosis; vasculitis; nephritis; arthritis; and osteoporosis.

10 28. A use of a binding agent in a medicament for the treatment of a disease associated with reduced osteopontin expression wherein said binding agent is an agonist of FGFR5 polypeptide function and wherein said binding agent is selected from the group consisting of: (a) a small molecule; (b) an antibody or antigen-binding fragment thereof; (c) a small chain antibody fragment (scFv); (d) a camelid heavy chain antibody (HCAb)
15 or heavy chain variable domain (V_{HH}) thereof; and (e) an FGFR5 ligand or FGFR5-binding fragment thereof.

29. The use of a binding agent of claim 28 wherein said binding agent specifically binds to a polypeptide selected from the group consisting of: (a) a
20 polypeptide encoded by a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 1-4 and 9 or a complement thereof; and (b) a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 5-8 and 13-15.

30. The use of a binding agent of claim 28 wherein said disease associated
25 with reduced osteopontin expression is selected from the group consisting of osteopetrosis.

31. A method for the treatment of a disease associated with elevated osteopontin expression, said method comprising the step of administering to a patient a
30 composition according to claims 1 or claim 6.

32. A method for the treatment of cancer in a patient, said method comprising the step of administering to said patient a composition according to claim 1 or claim 6 wherein said cancer is selected from the group consisting breast cancer, hepatocellular carcinoma, and colon cancer.

5

33. A method for the treatment of a bone disorder in a patient, said method comprising the step of administering to said patient a composition according to claim 1 or claim 6 wherein said bone disorder is selected from the group consisting osteoporosis and osteopetrosis.

10

34. A method for the treatment of an FGFR5-associated disorder in a patient, said method comprising the step of administering to said patient a composition according to claim 1 or claim 6.

15

35. A method for inhibiting the expression of osteopontin in a population of cells, comprising reducing the amount of a polypeptide in the cells, the polypeptide comprising an amino acid sequence selected from the group consisting of:

- (a) a sequence provided in SEQ ID NO: 5-8 and 13-15;
- (b) sequences having at least 75% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15
- (c) sequences having at least 90% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15; and
- (d) sequences having at least 95% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15.

25

36. A method for inhibiting the expression of osteopontin in a population of cells, comprising the step of inhibiting the activity of a polypeptide in said population of cells by administering a composition of claim 6 wherein said polypeptide comprising an amino acid sequence selected from the group consisting of:

- (a) a sequence provided in SEQ ID NO: 5-8 and 13-15;

30

(b) sequences having at least 75% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15;

(c) sequences having at least 90% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15; and

5 (d) sequences having at least 95% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15.

37. A method for treating a disorder characterized by an elevated level of osteopontin, comprising the step of administering a composition of 6 wherein said
10 composition comprises a binding agent that specifically to a polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) sequences provided in SEQ ID NO: 5-8 and 13-15;

(b) sequences having at least 75% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15;

15 (c) sequences having at least 90% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15; and

(d) sequences having at least 95% identity to a sequence provided in SEQ ID NO: 5-8 and 13-15.

20 38. A method for treating a disorder characterized by an elevated level of osteopontin, comprising administering a composition of claim 1 said composition comprises a modulator of FGFR5 gene expression that binds specifically to a polynucleotide comprising a sequence selected from the group consisting of:

(a) sequences provided in SEQ ID NO: 1-4 and 9;

25 (b) sequences having at least 75% identity to a sequence provided in SEQ ID NO: 1-4 and 9;

(c) sequences having at least 90% identity to a sequence provided in SEQ ID NO: 1-4 and 9; and

(d) sequences having at least 95% identity to a sequence provided in SEQ ID
30 NO: 1-4 and 9.

39. The method of any one of claims 35-38, wherein the disorder is selected from the group consisting of: cancer; multiple sclerosis; systemic lupus erythematosus; diabetes; rheumatoid arthritis; sarcoidosis; tuberculosis; kidney stones; atherosclerosis; vasculitis; nephritis; arthritis; and osteoporosis.

5

Abstract of the Disclosure

Isolated fibroblast growth factor receptor (FGFR5) polypeptides and polynucleotides encoding such polypeptides are provided. Also provided are modulators of FGFR5 gene expression and binding molecules that specifically bind to and agonize or antagonize FGFR5 polypeptide function. Specific binding molecules include antibodies, functional fragments thereof, as well as scFv and *Camelidae* heavy chain IgG that specifically bind to FGFR5 thereby modulating the activity of FGFR5 and, thus, are effective agents suitable for the treatment of diseases such as osteopontin-mediated autoimmune disease, such as systemic lupus erythematosus, bone disorders including osteoporosis and osteopetrosis, and cancers, including cellular carcinomas such as hepatocellular carcinomas.

1/11

Figure 1

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2/11

Fig. 2A

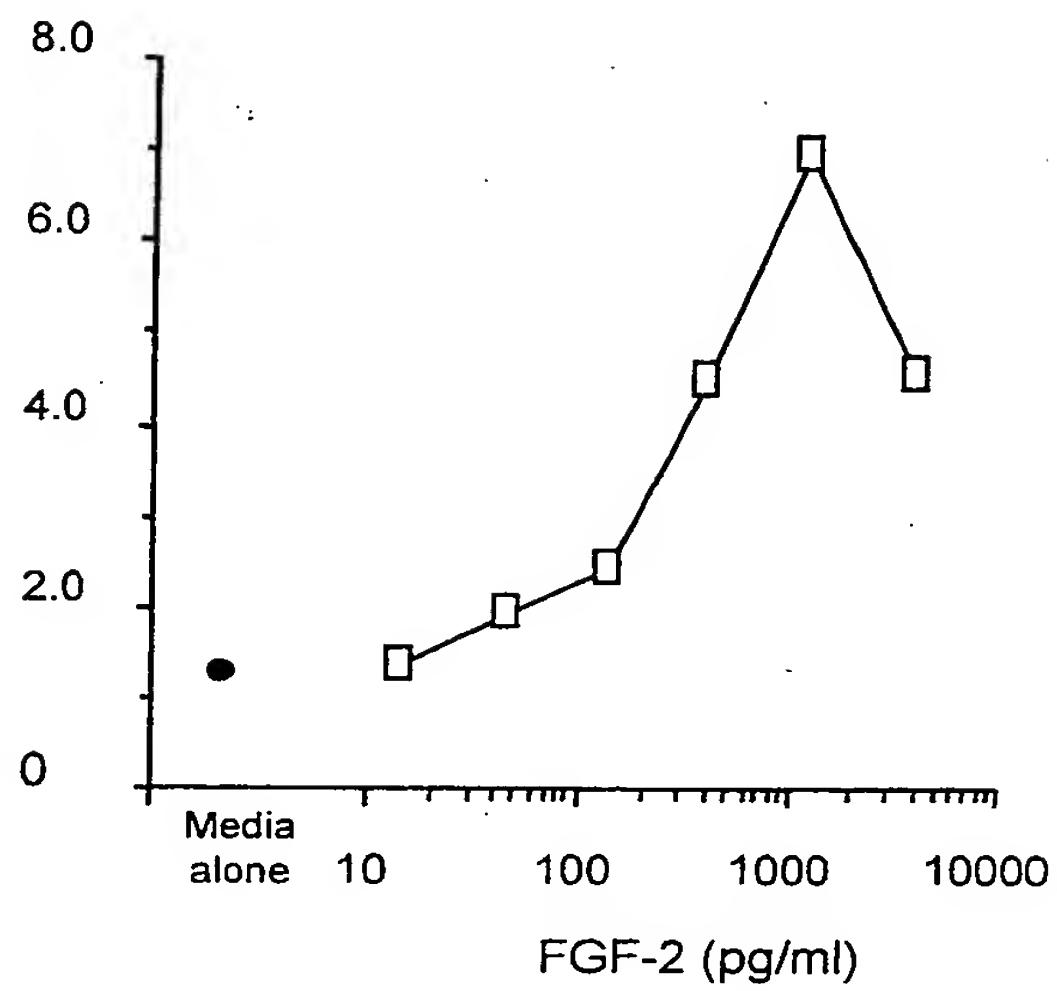
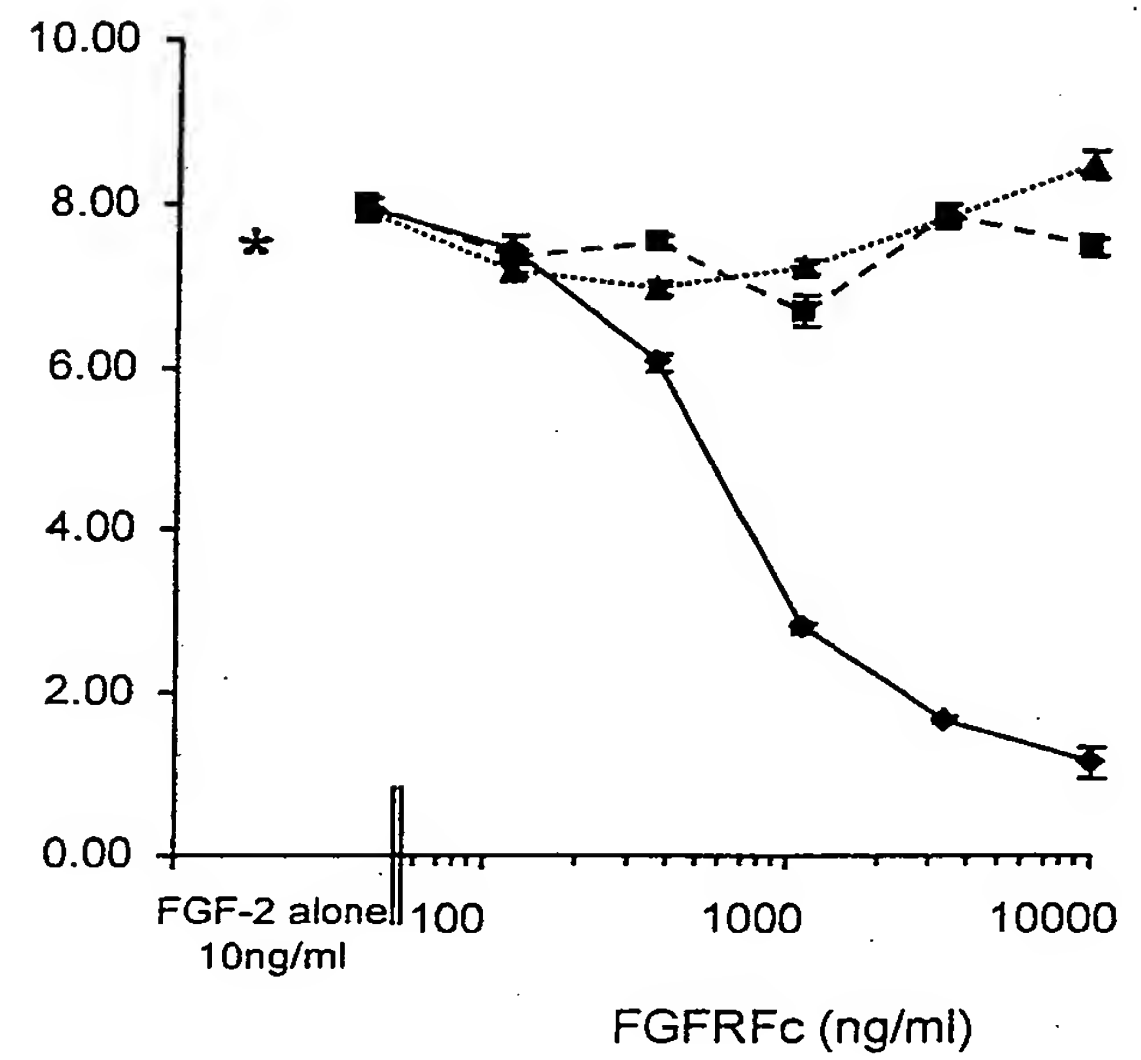


Fig. 2B



3/11

Fig. 3

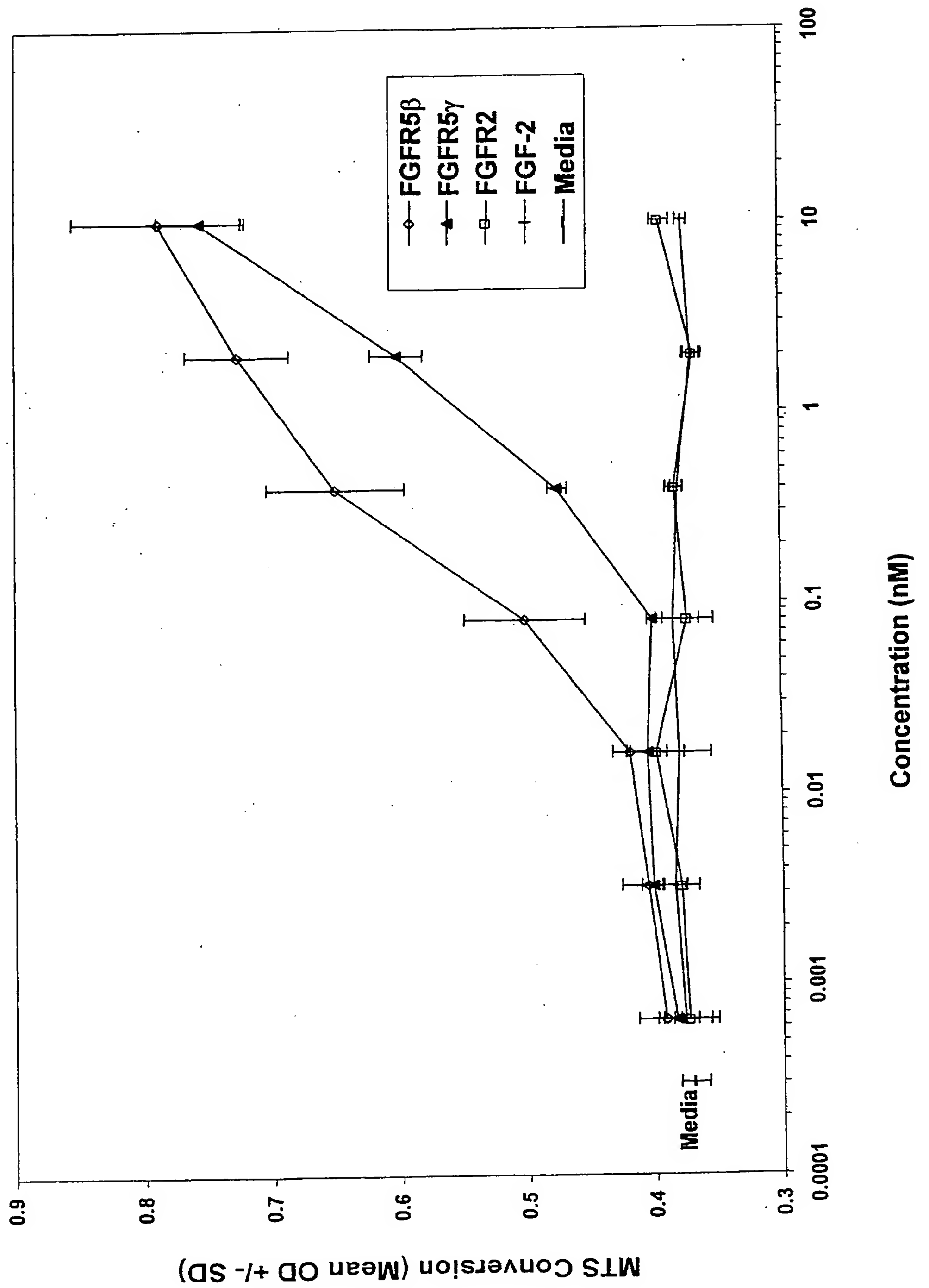


Fig. 4

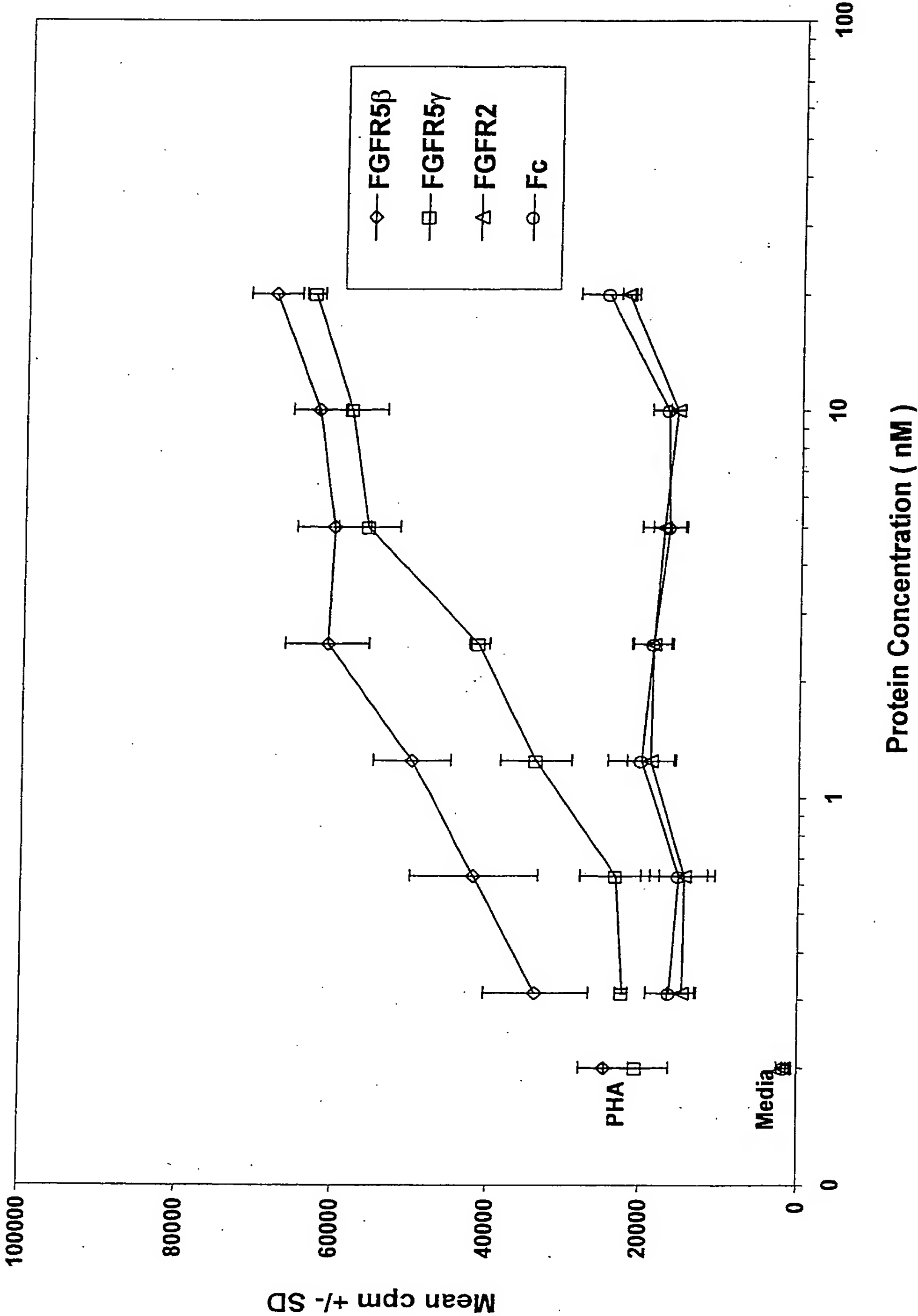
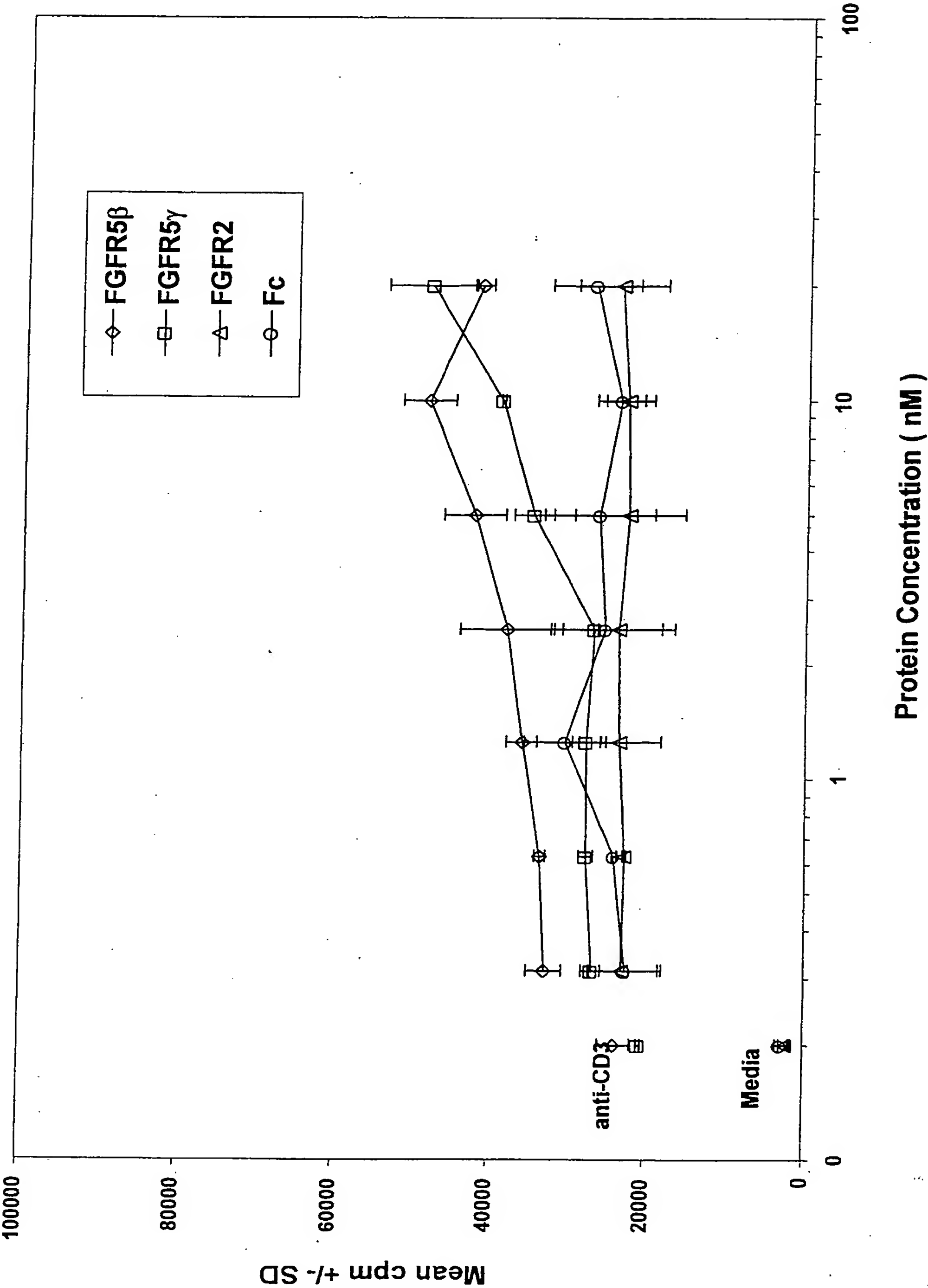


Fig. 5



6/11

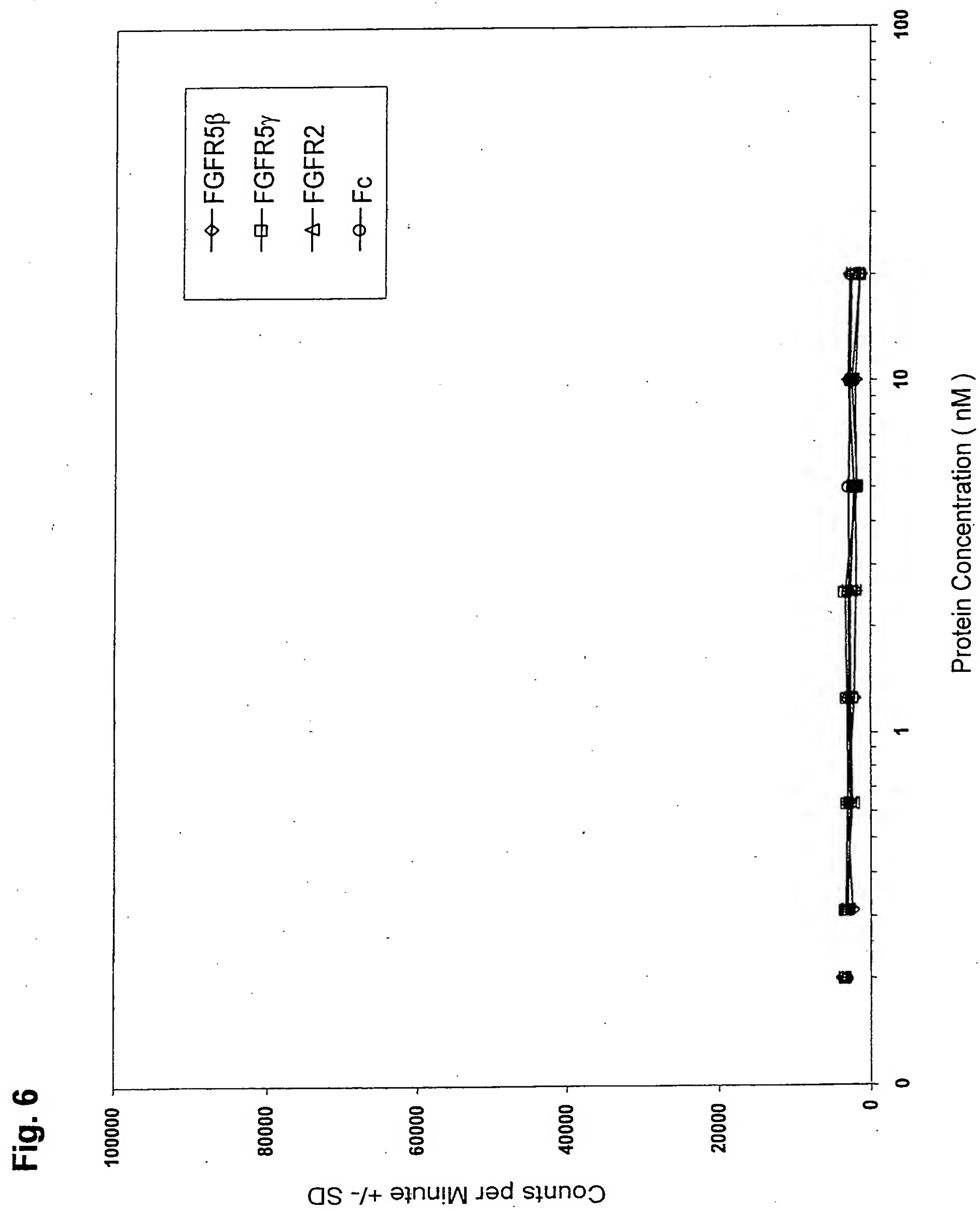
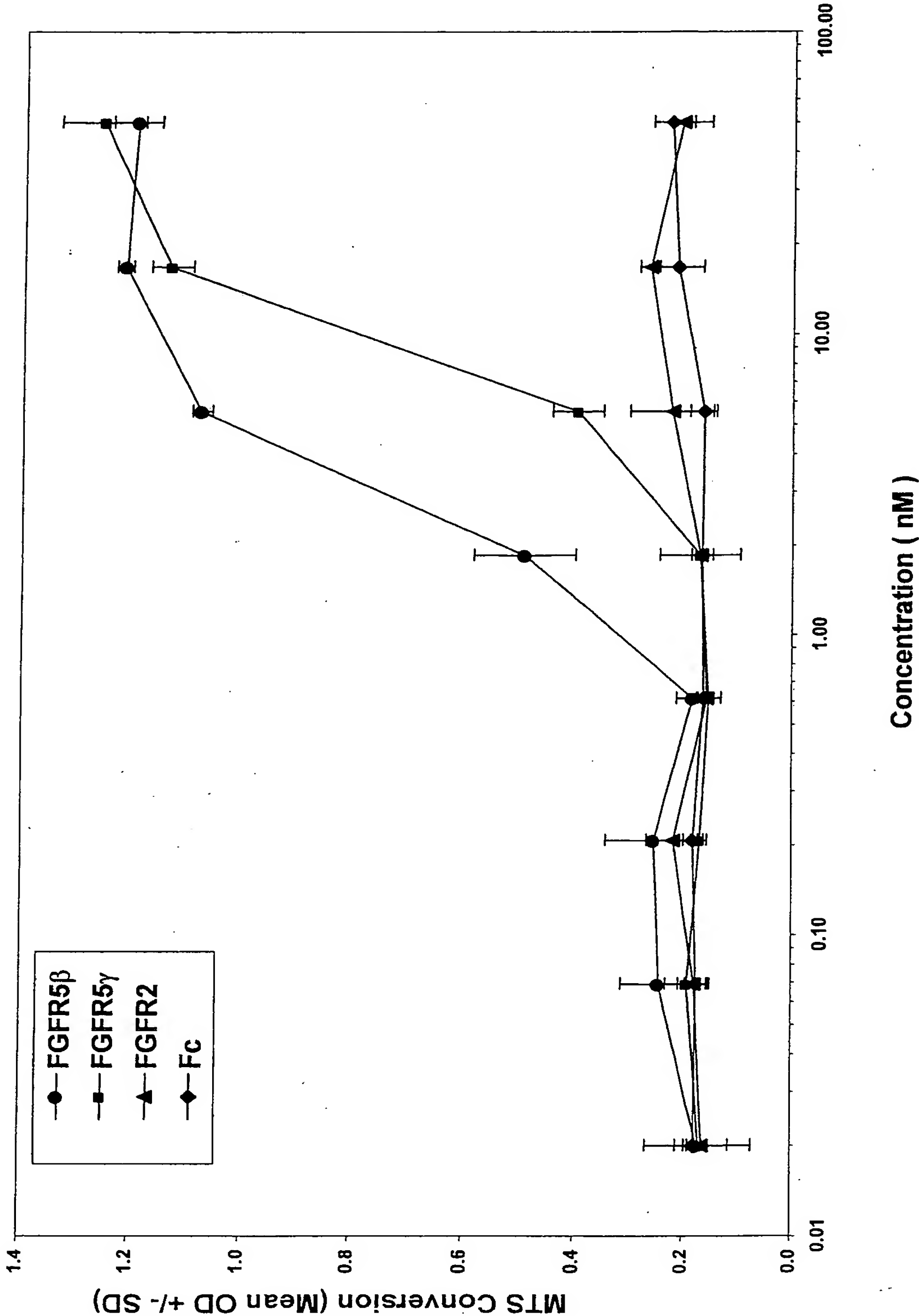


Fig. 7



8/11

Fig. 8

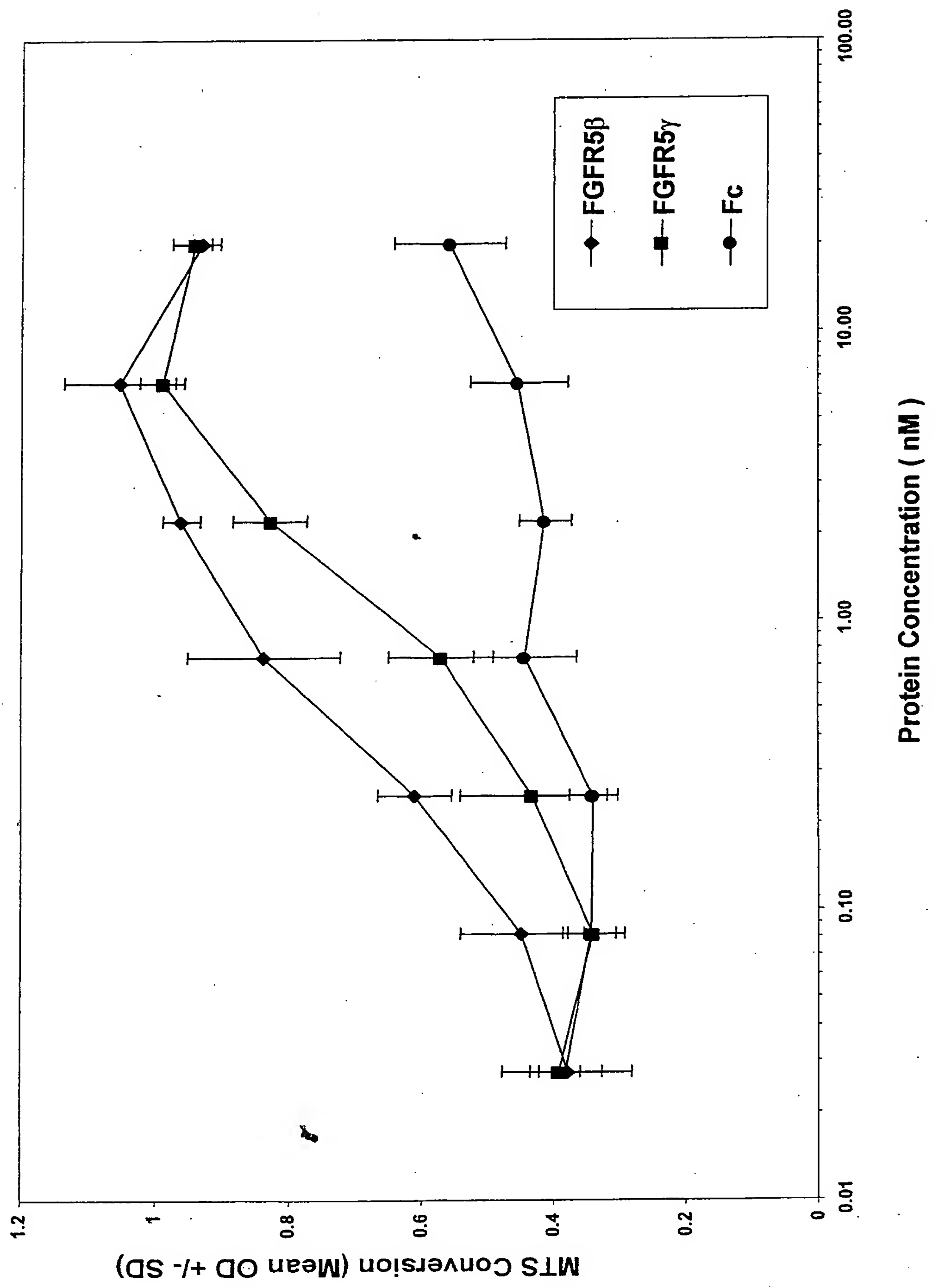
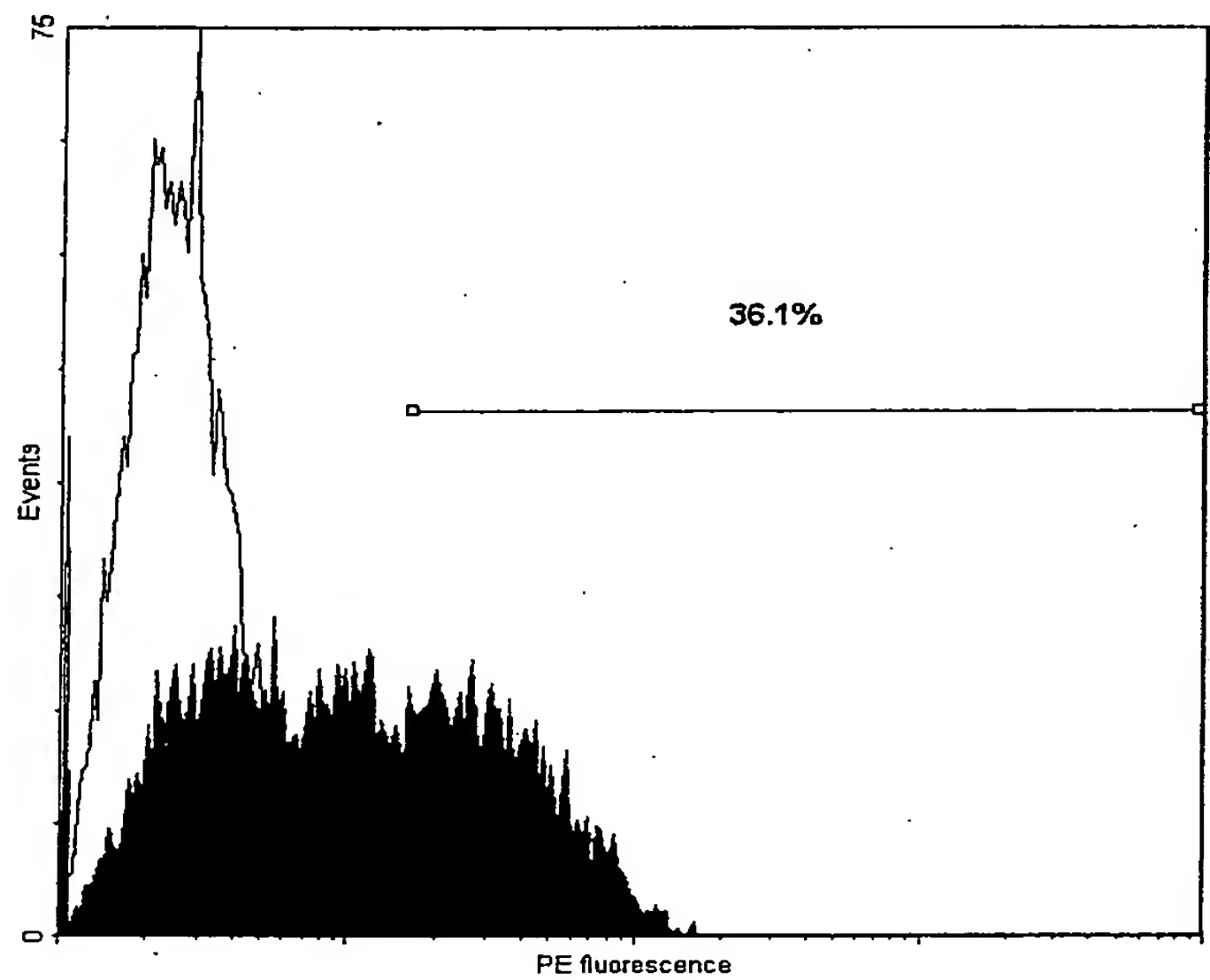


Fig. 9

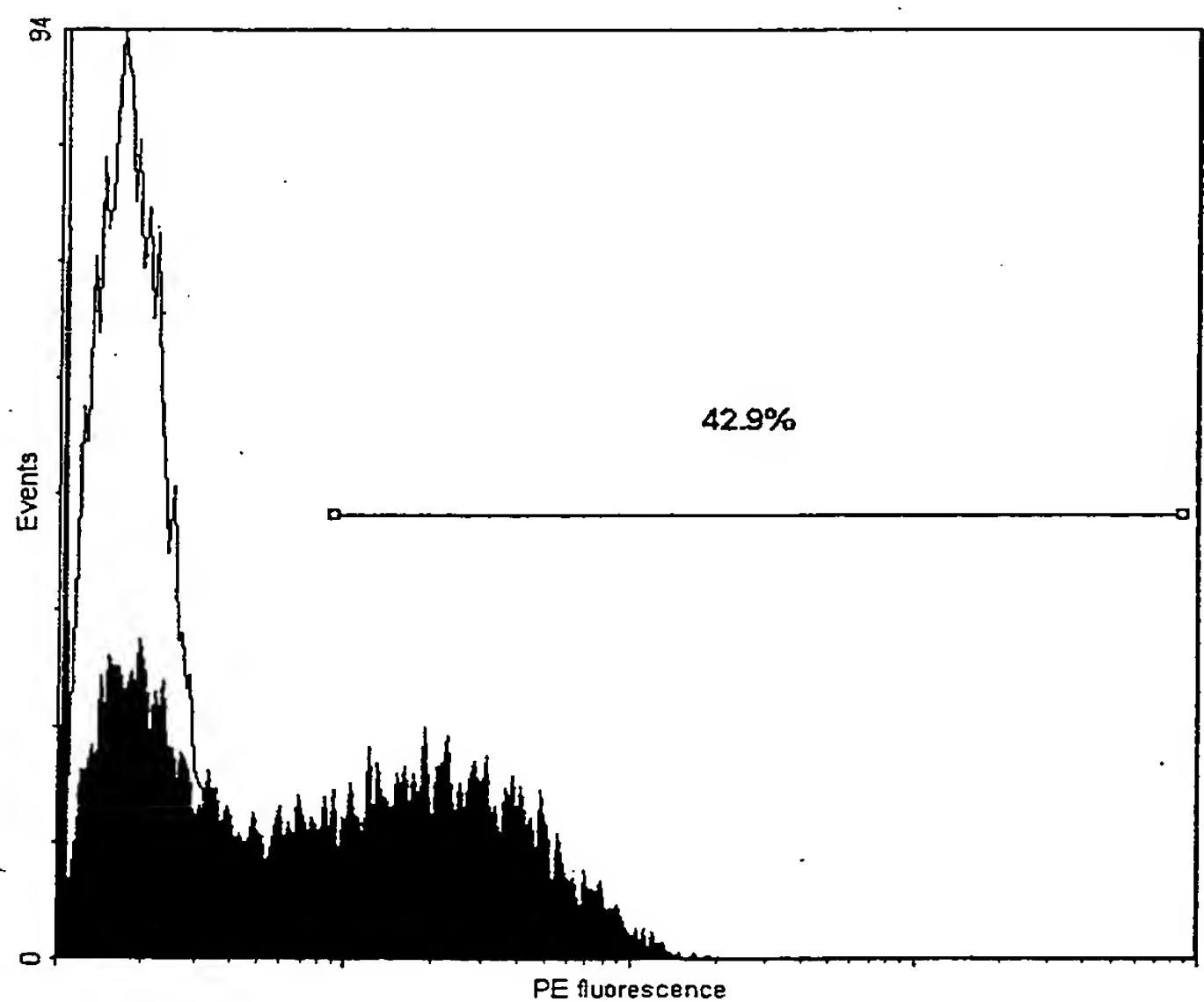
FGFR5 β -treated PBMC

Overlay Plot 1



FGFR5 γ -treated PBMC

Overlay Plot 2



10/11

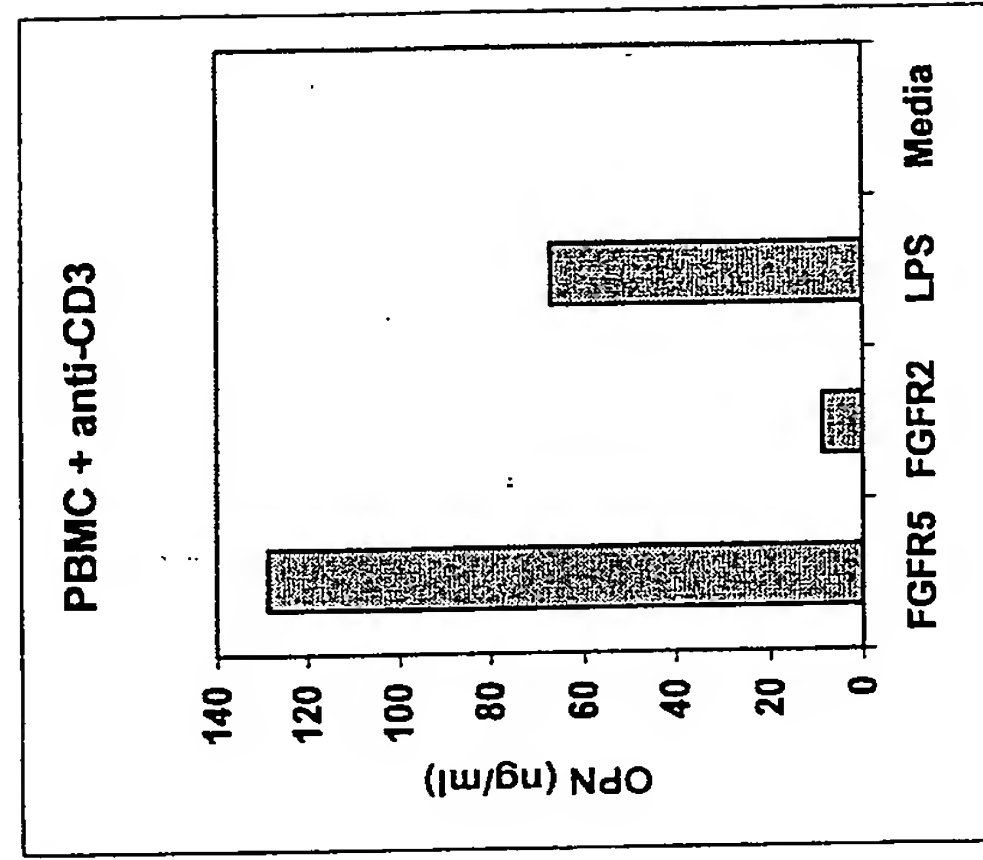
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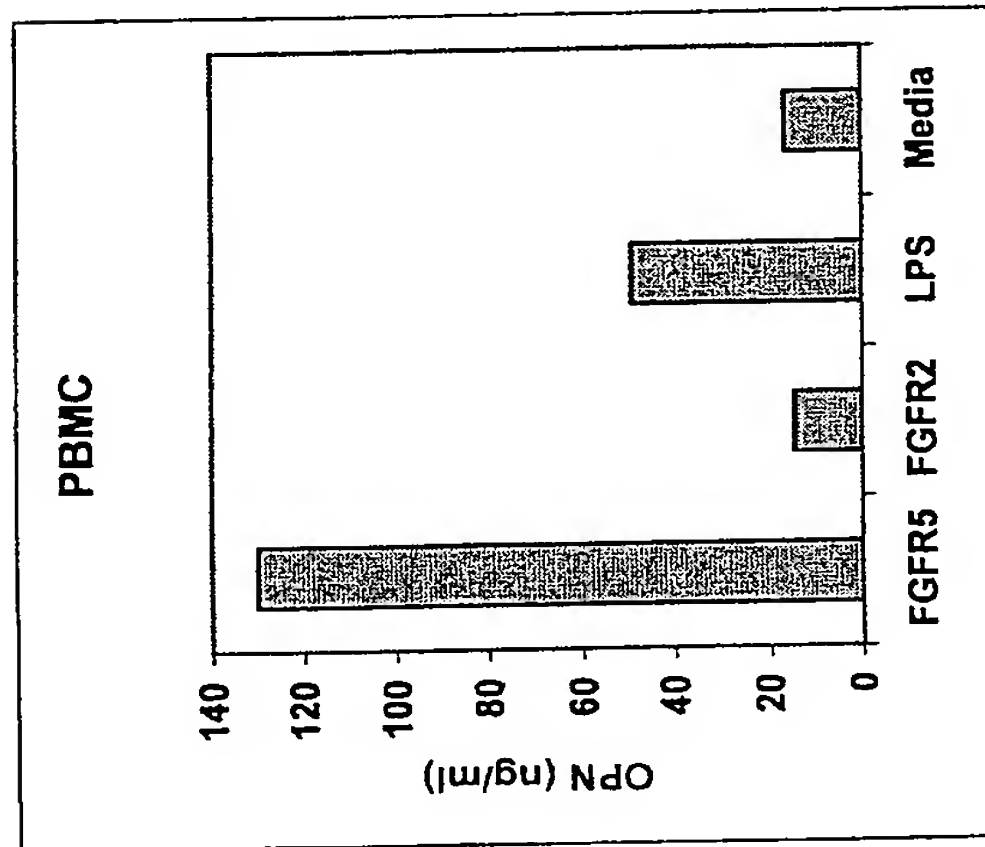
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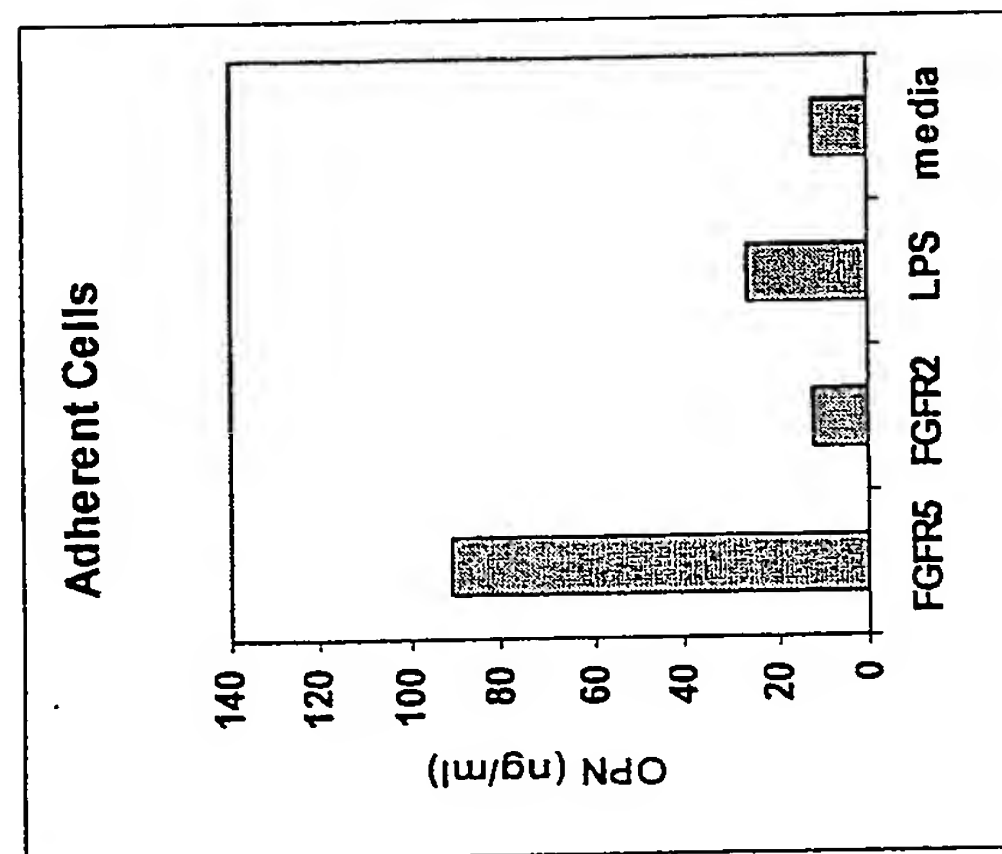
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A



In the application of **Genesis Research and Development Corporation**

Application No: **PCT/NZ030 0 1 0 5**

Filed: 27 May 2003

For: **FIBROBLAST GROWTH FACTOR RECEPTORS AND METHODS
FOR THEIR USE**

STATEMENT

International Searching Authority
Australian Patent Office

Sir

The undersigned verifies that, to the best of his knowledge, after making a comparison, the content of the computer readable sequence listing and the paper sequence listing as originally filed are the same.

Respectfully submitted



Jane Louise Calvert
BALDWIN SHELSTON WATERS

Date: 27 May 2003

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AND METHODS FOR THEIR USE

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ctgatggctc	ctacctcaac	aagctgctca	tctctcgggc	ccgccaggat	gatgctggca	1140
tgtacatctg	cctaggtgca	aataccatgg	gctacagttt	ccgtagcgcc	ttcctcactg	1200
tattaccaga	ccccaaacct	cctccagggc	ctcctatggc	ttcttcactg	tcattccaca	1260
gcctgccatg	gcctgtgggtg	atcggcatcc	cagctgggtgc	tgtcttcatc	ctaggcactg	1320
tgctgctctg	gctttgccag	accaagaaga	agccatgtgc	cccagcatct	acacttcctg	1380
tgccctggga	tcgtccccca	gggacatccc	gagaacgcag	tggtgacaag	gacctgcctt	1440
cattggctgt	gggcatatgt	gaggagcatg	gatccgcat	ggccccccag	cacatcctgg	1500
cctctggctc	aactgctggc	cccaagctgt	acccaagct	atacacagat	gtgcacacac	1560

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acacacatac acacacctgc actcacacgc tctcatgtgg agggcaaggt tcatcaacac 1620
cagcatgtcc actatcagtg ctaaatacag cgaatctcca agcactgtgt cctgaggtag 1680
gcatatgggg gccaaaggcaa caggttggga gaattgagaa caatggagga agagtatctt 1740
ag 1742

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<210> 4
 <211> 1004
 <212> DNA
 <213> Mouse

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<400> 4
gcggccgcga cccaggtcc ggacaggccg agatgacgcc gagccccctg ttgctgctcc 60
tgctgccgcc gctgctgctg ggggccttcc caccggccgc cgccgccga ggcccccaa 120
agatggcgga caaggtggtc ccacggcagg tggccggctg ggccgcactg tgcggctgca 180
gtgccagtgg agggggaccc gccgcgctg accatgtgga ccaaggatgg ccgcaccatc 240
cacagcggtt ggagccgctt ccgcgtgctg ccgcaggggc tgaagggtgaa gcaggtggag 300
cgggaggatg ccggcggtga cgtgtgcaag gccaccaacg gcttcggcag ccttagcgctc 360
aactacaccc tcgtcgtgct ggatgacatt agcccaggga aggagagcct ggggcccgcac 420
agctcctctg ggggtcaaga ggaccccgcc agccagcagt gggcacgacc gcgcttcaca 480
cagccctcca agatgaggcg ccgggtgatc gcacggcccg tgggtagctc cgtgcggctc 540
aagtgcgtgg ccagcgggca ccctcggccc gacatcacgt ggatgaagga cgaccaggcc 600
ttgacgcgcc cagaggccgc tgagcccagg aagaagaagt ggacactgag cctgaagaac 660
ctgcggccgg aggacagcgg caaatacacc tgccgcgtgt cgaaccgcgc gggcgccatc 720
aacgccacct acaaggtgga tgtgatccag cggaccggtt ccaagcccgt gctcacaggc 780
acgcaccccg tgaacacgac ggtggacttc ggggggacca cgtccttcca gtgcaagggtg 840
cgcagcgacg tgaagccggt gatccagtgg ctgaagcgcg tggagtacgg cgccgagggc 900
cgccacaact ccaccatcga tgtgggcggc cagaagtttg tgggtgctgc cacgggtgac 960
gtgtggtcgc ggcccgcgac ctctacctc aataagccgc tccc 1004

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<210> 5
 <211> 126
 <212> PRT
 <213> Mouse

<220>
 <221> VARIANT
 <222> (1)...(126)
 <223> Xaa = Any Amino Acid

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<400> 5
Val Asp Phe Gly Gly Thr Thr Ser Phe Gln Cys Lys Val Arg Ser Asp
1 5 10 15
Val Lys Pro Val Ile Gln Trp Leu Lys Arg Val Glu Tyr Gly Ser Glu
20 25 30
Gly Arg His Asn Ser Thr Ile Asp Val Gly Gly Gln Lys Phe Val Val
35 40 45
Leu Pro Thr Gly Asp Val Trp Ser Arg Pro Asp Gly Ser Tyr Leu Asn
50 55 60
Lys Leu Leu Ile Ser Arg Ala Arg Gln Asp Asp Ala Gly Met Tyr Ile
65 70 75 80
Cys Leu Gly Ala Asn Thr Met Gly Tyr Ser Phe Arg Ser Ala Phe Leu
85 90 95
Thr Val Leu Pro Asp Pro Lys Pro Pro Gly Pro Pro Met Ala Ser Ser
100 105 110
Ser Ser Ser Thr Ser Leu Pro Trp Pro Val Xaa Gly Ile Pro
115 120 125

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<210> 6
 <211> 529
 <212> PRT
 <213> Mouse

<400> 6

Met	Thr	Arg	Ser	Pro	Ala	Leu	Leu	Leu	Leu	Leu	Leu	Gly	Ala	Leu	Pro
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Ser	Ala	Glu	Ala	Ala	Arg	Gly	Pro	Pro	Arg	Met	Ala	Asp	Lys	Val	Val
			20					25					30		
Pro	Arg	Gln	Val	Ala	Arg	Leu	Gly	Arg	Thr	Val	Arg	Leu	Gln	Cys	Pro
		35					40					45			
Val	Glu	Gly	Asp	Pro	Pro	Pro	Leu	Thr	Met	Trp	Thr	Lys	Asp	Gly	Arg
	50					55					60				
Thr	Ile	His	Ser	Gly	Trp	Ser	Arg	Phe	Arg	Val	Leu	Pro	Gln	Gly	Leu
65					70					75					80
Lys	Val	Lys	Glu	Val	Glu	Ala	Glu	Asp	Ala	Gly	Val	Tyr	Val	Cys	Lys
			85						90					95	
Ala	Thr	Asn	Gly	Phe	Gly	Ser	Leu	Ser	Val	Asn	Tyr	Thr	Leu	Ile	Ile
			100					105					110		
Met	Asp	Asp	Ile	Ser	Pro	Gly	Lys	Glu	Ser	Pro	Gly	Pro	Gly	Gly	Ser
		115					120					125			
Ser	Gly	Gly	Gln	Glu	Asp	Pro	Ala	Ser	Gln	Gln	Trp	Ala	Arg	Pro	Arg
	130					135					140				
Phe	Thr	Gln	Pro	Ser	Lys	Met	Arg	Arg	Arg	Val	Ile	Ala	Arg	Pro	Val
145					150					155					160
Gly	Ser	Ser	Val	Arg	Leu	Lys	Cys	Val	Ala	Ser	Gly	His	Pro	Arg	Pro
			165					170						175	
Asp	Ile	Met	Trp	Met	Lys	Asp	Asp	Gln	Thr	Leu	Thr	His	Leu	Glu	Ala
			180					185					190		
Ser	Glu	His	Arg	Lys	Lys	Lys	Trp	Thr	Leu	Ser	Leu	Lys	Asn	Leu	Lys
		195					200					205			
Pro	Glu	Asp	Ser	Gly	Lys	Tyr	Thr	Cys	Arg	Val	Ser	Asn	Lys	Ala	Gly
	210					215					220				
Ala	Ile	Asn	Ala	Thr	Tyr	Lys	Val	Asp	Val	Ile	Gln	Arg	Thr	Arg	Ser
225					230					235					240
Lys	Pro	Val	Leu	Thr	Gly	Thr	His	Pro	Val	Asn	Thr	Thr	Val	Asp	Phe
				245					250					255	
Gly	Gly	Thr	Thr	Ser	Phe	Gln	Cys	Lys	Val	Arg	Ser	Asp	Val	Lys	Pro
			260					265					270		
Val	Ile	Gln	Trp	Leu	Lys	Arg	Val	Glu	Tyr	Gly	Ser	Glu	Gly	Arg	His
		275					280					285			
Asn	Ser	Thr	Ile	Asp	Val	Gly	Gly	Gln	Lys	Phe	Val	Val	Leu	Pro	Thr
	290					295					300				
Gly	Asp	Val	Trp	Ser	Arg	Pro	Asp	Gly	Ser	Tyr	Leu	Asn	Lys	Leu	Leu
305					310					315					320
Ile	Ser	Arg	Ala	Arg	Gln	Asp	Asp	Ala	Gly	Met	Tyr	Ile	Cys	Leu	Gly
			325						330					335	
Ala	Asn	Thr	Met	Gly	Tyr	Ser	Phe	Arg	Ser	Ala	Phe	Leu	Thr	Val	Leu
			340					345					350		
Pro	Asp	Pro	Lys	Pro	Pro	Gly	Pro	Pro	Met	Ala	Ser	Ser	Ser	Ser	Ser
		355					360					365			
Thr	Ser	Leu	Pro	Trp	Pro	Val	Val	Ile	Gly	Ile	Pro	Ala	Gly	Ala	Val
	370					375					380				
Phe	Ile	Leu	Gly	Thr	Val	Leu	Leu	Trp	Leu	Cys	Gln	Thr	Lys	Lys	Lys
385					390					395					400
Pro	Cys	Ala	Pro	Ala	Ser	Thr	Leu	Pro	Val	Pro	Gly	His	Arg	Pro	Pro
			405						410					415	
Gly	Thr	Ser	Arg	Glu	Arg	Ser	Gly	Asp	Lys	Asp	Leu	Pro	Ser	Leu	Ala
			420					425					430		
Val	Gly	Ile	Cys	Glu	Glu	His	Gly	Ser	Ala	Met	Ala	Pro	Gln	His	Ile
		435					440					445			
Leu	Ala	Ser	Gly	Ser	Thr	Ala	Gly	Pro	Lys	Leu	Tyr	Pro	Lys	Leu	Tyr
	450					455					460				
Thr	Asp	Val	His	Thr	His	Thr	His	Thr	His	Thr	Cys	Thr	His	Thr	Leu

465 470 475 480
 Ser Cys Gly Gly Gln Gly Ser Ser Thr Pro Ala Cys Pro Leu Ser Val
 485 490 495
 Leu Asn Thr Ala Asn Leu Gln Ala Leu Cys Pro Glu Val Gly Ile Trp
 500 505 510
 Gly Pro Arg Gln Gln Val Gly Arg Ile Glu Asn Asn Gly Gly Arg Val
 515 520 525
 Ser

<210> 7
 <211> 439
 <212> PRT
 <213> Mouse

<400> 7
 Met Thr Arg Ser Pro Ala Leu Leu Leu Leu Leu Leu Gly Ala Leu Pro
 1 5 10 15
 Ser Ala Glu Ala Ala Arg Asp Asp Ile Ser Pro Gly Lys Glu Ser Pro
 20 25 30
 Gly Pro Gly Gly Ser Ser Gly Gly Gln Glu Asp Pro Ala Ser Gln Gln
 35 40 45
 Trp Ala Arg Pro Arg Phe Thr Gln Pro Ser Lys Met Arg Arg Arg Val
 50 55 60
 Ile Ala Arg Pro Val Gly Ser Ser Val Arg Leu Lys Cys Val Ala Ser
 65 70 75 80
 Gly His Pro Arg Pro Asp Ile Met Trp Met Lys Asp Asp Gln Thr Leu
 85 90 95
 Thr His Leu Glu Ala Ser Glu His Arg Lys Lys Lys Trp Thr Leu Ser
 100 105 110
 Leu Lys Asn Leu Lys Pro Glu Asp Ser Gly Lys Tyr Thr Cys Arg Val
 115 120 125
 Ser Asn Lys Ala Gly Ala Ile Asn Ala Thr Tyr Lys Val Asp Val Ile
 130 135 140
 Gln Arg Thr Arg Ser Lys Pro Val Leu Thr Gly Thr His Pro Val Asn
 145 150 155 160
 Thr Thr Val Asp Phe Gly Gly Thr Thr Ser Phe Gln Cys Lys Val Arg
 165 170 175
 Ser Asp Val Lys Pro Val Ile Gln Trp Leu Lys Arg Val Glu Tyr Gly
 180 185 190
 Ser Glu Gly Arg His Asn Ser Thr Ile Asp Val Gly Gly Gln Lys Phe
 195 200 205
 Val Val Leu Pro Thr Gly Asp Val Trp Ser Arg Pro Asp Gly Ser Tyr
 210 215 220
 Leu Asn Lys Leu Leu Ile Ser Arg Ala Arg Gln Asp Asp Ala Gly Met
 225 230 235 240
 Tyr Ile Cys Leu Gly Ala Asn Thr Met Gly Tyr Ser Phe Arg Ser Ala
 245 250 255
 Phe Leu Thr Val Leu Pro Asp Pro Lys Pro Pro Pro Gly Pro Pro Met
 260 265 270
 Ala Ser Ser Ser Ser Ser Thr Ser Leu Pro Trp Pro Val Val Ile Gly
 275 280 285
 Ile Pro Ala Gly Ala Val Phe Ile Leu Gly Thr Val Leu Leu Trp Leu
 290 295 300
 Cys Gln Thr Lys Lys Lys Pro Cys Ala Pro Ala Ser Thr Leu Pro Val
 305 310 315 320
 Pro Gly His Arg Pro Pro Gly Thr Ser Arg Glu Arg Ser Gly Asp Lys
 325 330 335
 Asp Leu Pro Ser Leu Ala Val Gly Ile Cys Glu Glu His Gly Ser Ala
 340 345 350
 Met Ala Pro Gln His Ile Leu Ala Ser Gly Ser Thr Ala Gly Pro Lys

355 360 365
 Leu Tyr Pro Lys Leu Tyr Thr Asp Val His Thr His Thr His Thr His
 370 375 380
 Thr Cys Thr His Thr Leu Ser Cys Gly Gly Gln Gly Ser Ser Thr Pro
 385 390 395 400
 Ala Cys Pro Leu Ser Val Leu Asn Thr Ala Asn Leu Gln Ala Leu Cys
 405 410 415
 Pro Glu Val Gly Ile Trp Gly Pro Arg Gln Gln Val Gly Arg Ile Glu
 420 425 430
 Asn Asn Gly Gly Arg Val Ser
 435

<210> 8
 <211> 322
 <212> PRT
 <213> Mouse

<400> 8
 Arg Arg Ala Pro Cys Cys Cys Ser Cys Cys Arg Arg Cys Cys Trp Gly
 1 5 10 15
 Pro Ser His Arg Pro Pro Pro Pro Glu Ala Pro Gln Arg Trp Arg Thr
 20 25 30
 Arg Trp Ser His Gly Arg Trp Pro Ala Gly Pro His Cys Ala Ala Ala
 35 40 45
 Val Pro Val Glu Gly Asp Pro Pro Pro Leu Thr Met Trp Thr Lys Asp
 50 55 60
 Gly Arg Thr Ile His Ser Gly Trp Ser Arg Phe Arg Val Leu Pro Gln
 65 70 75 80
 Gly Leu Lys Val Lys Gln Val Glu Arg Glu Asp Ala Gly Val Tyr Val
 85 90 95
 Cys Lys Ala Thr Asn Gly Phe Gly Ser Leu Ser Val Asn Tyr Thr Leu
 100 105 110
 Val Val Leu Asp Asp Ile Ser Pro Gly Lys Glu Ser Leu Gly Pro Asp
 115 120 125
 Ser Ser Ser Gly Gly Gln Glu Asp Pro Ala Ser Gln Gln Trp Ala Arg
 130 135 140
 Pro Arg Phe Thr Gln Pro Ser Lys Met Arg Arg Arg Val Ile Ala Arg
 145 150 155 160
 Pro Val Gly Ser Ser Val Arg Leu Lys Cys Val Ala Ser Gly His Pro
 165 170 175
 Arg Pro Asp Ile Thr Trp Met Lys Asp Asp Gln Ala Leu Thr Arg Pro
 180 185 190
 Glu Ala Ala Glu Pro Arg Lys Lys Lys Trp Thr Leu Ser Leu Lys Asn
 195 200 205
 Leu Arg Pro Glu Asp Ser Gly Lys Tyr Thr Cys Arg Val Ser Asn Arg
 210 215 220
 Ala Gly Ala Ile Asn Ala Thr Tyr Lys Val Asp Val Ile Gln Arg Thr
 225 230 235 240
 Arg Ser Lys Pro Val Leu Thr Gly Thr His Pro Val Asn Thr Thr Val
 245 250 255
 Asp Phe Gly Gly Thr Thr Ser Phe Gln Cys Lys Val Arg Ser Asp Val
 260 265 270
 Lys Pro Val Ile Gln Trp Leu Lys Arg Val Glu Tyr Gly Ala Glu Gly
 275 280 285
 Arg His Asn Ser Thr Ile Asp Val Gly Gly Gln Lys Phe Val Val Leu
 290 295 300
 Pro Thr Gly Asp Val Trp Ser Arg Pro Asp Gly Ser Tyr Leu Asn Lys
 305 310 315 320
 Pro Leu

<210> 9
 <211> 1423
 <212> DNA
 <213> Mouse

<400> 9
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 gatgaccaga ccttgacgca tctagaggct agtgaacaca gaaagaagaa gtggacactg 120
 agcttgaaga acctgaagcc tgaagacagt ggcaagtaca cgtgccgtgt atctaacaag 180
 gccggtgcc tcaacgccac ctacaaagtgt gatgtaatcc gtgagtgggt ggtctgtggt 240
 aggacagggg cccgtgggtgc ctaaaactgt gctgacatgt ttgtttttcc ttggcttaga 300
 gcggactcgt tccaagcctg tgctcacagg gacacaccct gtgaacacaa cgggtggactt 360
 cgggtgggaca acgtccttcc agtgcaagggt gcgcagtgc gtgaagcctg tgatccagtg 420
 gctgaagcgg gtggagtacg gctccgaggg acgccacaac tccaccattg atgtgggtgg 480
 ccagaagttt gtggtgttgc ccacgggtga tgtgtggtca cggcctgatg gctcctacct 540
 caacaagctg ctcatctctc gggcccgcga ggatgatgct ggcatgtaca tctgcctagg 600
 tgcaaatacc atgggctaca gtttccgtag cgccttctc actgtattac caggtgtgtg 660
 tgtgggctgc ccaccccatg tttactctca gtctctacca ttggtctggg ctgtcctggg 720
 gttccccaat gtccacttag caagtggggc ctccctatcc ttttcccttc gttgtgggtt 780
 atccttgctt catagggagt tcaggggtgc tgcccatata gttcacattt gggctgggtt 840
 cccattaat atagggacat tctgtccctt actcttcttc ttaatctctc ttgcagacct 900
 caaacctcca gggcctccta tggcttcttc atcgtcatcc acaagcctgc catggcctgt 960
 ggtgatcggc atcccagctg gtgctgtctt catcctaggc actgtgctgc tctggctttg 1020
 ccagaccaag aagaagccat gtgccccagc atctacactt cctgtgectg ggcacgtcc 1080
 cccagggaca tcccgagAAC gcagtgggtga caaggacctg ccttcattgg ctgtgggcat 1140
 atgtgaggag catggatccg ccatggcccc ccagcacatc ctggcctctg gctcaactgc 1200
 tggccccaag ctgtaccca agctatacac agatgtgcac acacacacac atacacacac 1260
 ctgcactcac acgtctctat gtggagggca aggttcatca acaccagcat gtccactatc 1320
 agtgctaaat acagcgaatc tccaagcact gtgtcctgag gtaggcatat gggggccaag 1380
 gcaacagggtt gggagaattg agaacaatgg aggaagagta tct 1423

<210> 10
 <211> 35
 <212> DNA
 <213> Mouse

<400> 10
 cgggatccag gccatggcag gcttgtggat gacga 35

<210> 11
 <211> 37
 <212> DNA
 <213> Mouse

<400> 11
 ccgctcgagt agatactctt cctccattgt tctcatt 37

<210> 12
 <211> 18
 <212> DNA
 <213> Mouse

<400> 12
 ctgtgaggct caagtgtg 18

<210> 13
 <211> 373
 <212> PRT
 <213> Mouse

<400> 13

Met Thr Arg Ser Pro Ala Leu Leu Leu Leu Leu Leu Gly Ala Leu Pro
 1 5 10 15
 Ser Ala Glu Ala Ala Arg Gly Pro Pro Arg Met Ala Asp Lys Val Val
 20 25 30
 Pro Arg Gln Val Ala Arg Leu Gly Arg Thr Val Arg Leu Gln Cys Pro
 35 40 45
 Val Glu Gly Asp Pro Pro Pro Leu Thr Met Trp Thr Lys Asp Gly Arg
 50 55 60
 Thr Ile His Ser Gly Trp Ser Arg Phe Arg Val Leu Pro Gln Gly Leu
 65 70 75 80
 Lys Val Lys Glu Val Glu Ala Glu Asp Ala Gly Val Tyr Val Cys Lys
 85 90 95
 Ala Thr Asn Gly Phe Gly Ser Leu Ser Val Asn Tyr Thr Leu Ile Ile
 100 105 110
 Met Asp Asp Ile Ser Pro Gly Lys Glu Ser Pro Gly Pro Gly Gly Ser
 115 120 125
 Ser Gly Gly Gln Glu Asp Pro Ala Ser Gln Gln Trp Ala Arg Pro Arg
 130 135 140
 Phe Thr Gln Pro Ser Lys Met Arg Arg Arg Val Ile Ala Arg Pro Val
 145 150 155 160
 Gly Ser Ser Val Arg Leu Lys Cys Val Ala Ser Gly His Pro Arg Pro
 165 170 175
 Asp Ile Met Trp Met Lys Asp Asp Gln Thr Leu Thr His Leu Glu Ala
 180 185 190
 Ser Glu His Arg Lys Lys Lys Trp Thr Leu Ser Leu Lys Asn Leu Lys
 195 200 205
 Pro Glu Asp Ser Gly Lys Tyr Thr Cys Arg Val Ser Asn Lys Ala Gly
 210 215 220
 Ala Ile Asn Ala Thr Tyr Lys Val Asp Val Ile Gln Arg Thr Arg Ser
 225 230 235 240
 Lys Pro Val Leu Thr Gly Thr His Pro Val Asn Thr Thr Val Asp Phe
 245 250 255
 Gly Gly Thr Thr Ser Phe Gln Cys Lys Val Arg Ser Asp Val Lys Pro
 260 265 270
 Val Ile Gln Trp Leu Lys Arg Val Glu Tyr Gly Ser Glu Gly Arg His
 275 280 285
 Asn Ser Thr Ile Asp Val Gly Gly Gln Lys Phe Val Val Leu Pro Thr
 290 295 300
 Gly Asp Val Trp Ser Arg Pro Asp Gly Ser Tyr Leu Asn Lys Leu Leu
 305 310 315 320
 Ile Ser Arg Ala Arg Gln Asp Asp Ala Gly Met Tyr Ile Cys Leu Gly
 325 330 335
 Ala Asn Thr Met Gly Tyr Ser Phe Arg Ser Ala Phe Leu Thr Val Leu
 340 345 350
 Pro Asp Pro Lys Pro Pro Gly Pro Pro Met Ala Ser Ser Ser Ser
 355 360 365
 Thr Ser Leu Pro Trp
 370

<210> 14
 <211> 135
 <212> PRT
 <213> Mouse

<400> 14
 Cys Gln Thr Lys Lys Lys Pro Cys Ala Pro Ala Ser Thr Leu Pro Val
 1 5 10 15
 Pro Gly His Arg Pro Pro Gly Thr Ser Arg Glu Arg Ser Gly Asp Lys
 20 25 30
 Asp Leu Pro Ser Leu Ala Val Gly Ile Cys Glu Glu His Gly Ser Ala
 35 40 45

Met Ala Pro Gln His Ile Leu Ala Ser Gly Ser Thr Ala Gly Pro Lys
 50 55 60
 Leu Tyr Pro Lys Leu Tyr Thr Asp Val His Thr His Thr His Thr His
 65 70 75 80
 Thr Cys Thr His Thr Leu Ser Cys Gly Gly Gln Gly Ser Ser Thr Pro
 85 90 95
 Ala Cys Pro Leu Ser Val Leu Asn Thr Ala Asn Leu Gln Ala Leu Cys
 100 105 110
 Pro Glu Val Gly Ile Trp Gly Pro Arg Gln Gln Val Gly Arg Ile Glu
 115 120 125
 Asn Asn Gly Gly Arg Val Ser
 130 135

<210> 15
 <211> 37
 <212> PRT
 <213> Mouse

<400> 15

Arg Val Glu Tyr Gly Ser Glu Gly Arg His Asn Ser Thr Ile Asp Val
 1 5 10 15
 Gly Gly Gln Lys Phe Val Val Leu Pro Thr Gly Asp Val Trp Ser Arg
 20 25 30
 Pro Asp Gly Ser Tyr
 35